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Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782

Susan N. Rosenzweig and Kenneth A. Souza

September 1978

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Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782

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National Aeronautics and
Space Administration

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PREFACE

The United States is continuing to enjoy substantial international cooperation in the area of space life sciences. Four months after the successful completion of the Apollo-Soyuz Test Project in which joint life sciences experimentation took place, the Soviet Union launched an unmanned dedicated biological satellite which, for the first time, carried an assortment of biological experiments designed and developed by the United States. The multi-national payload of this mission originated from scientists in Czechoslovakia, Hungary, Poland, Rumania, and France as well as the USSR and the US. This first joint mission began on November 25, 1975, with the launch and subsequent 19.5-day flight of Cosmos 782.

Cooperation between the US and USSR in the area of space biology and medicine began in 1971 with the signing of the US/USSR Science and Applications Agreement. A Joint US/USSR Working Group on Space Biology and Medicine was established and met periodically to exchange information obtained during spaceflights and to discuss problems and areas of mutual scientific interest. During the Fifth Meeting of the Joint US/USSR Working Group on Space Biology and Medicine in Tashkent, USSR, October 1974, the Soviets offered their American colleagues the opportunity to fly US biological experiments on board one or more Soviet spacecraft. The offer was accepted and a group of 11 experiments was selected and flown on Cosmos 782. Later, at the Sixth Joint US/USSR Working Group Meeting on Space Biology and Medicine in San Francisco, August 1975, the Soviets offered to fly additional US experiments on another unmanned biological satellite scheduled for launch during the summer of 1977.

Data on the effects of weightlessness, artificial gravity, and cosmic radiation on the biological processes at different developmental levels of plants and animals were obtained from the experiments and investigations flown on Cosmos 782. Specifically, four US experiments were designed to study the effects of weightlessness on cellular and physiological mechanisms in plants and fish and to evaluate the ionizing cosmic radiation encountered during the flight. The remaining seven US experiments, performed on tissue obtained from Soviet animals, were designed to investigate the morphological and biochemical changes to animals exposed to prolonged spaceflight. Rats, for example, were used to investigate changes observed in red blood cell life span and bone growth - fish, for changes in their vestibular system. The observations made here related to similar observations made on both astronauts and cosmonauts.

As evidenced by the scientific results obtained, the Cosmos 782 mission has made a substantial contribution to space biology and medicine. In addition, the Joint US/USSR Biological Satellite Program has established a level of international cooperation rarely achieved in science or other fields. It has been a great pleasure to take part in this program and, on behalf of all members of the NASA and the scientific community who

participated in the Cosmos 782 mission, I would like to extend our sincere thanks to the Soviet government and the Soviet Academy of Sciences for making our participation possible and to our Soviet colleagues for their superb assistance in the execution of the program.

Lawrence C. Chambers
Director, Cosmos 782 Program
NASA Headquarters

US EXPERIMENTS FLOWN ON

COSMOS 782

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ABSTRACT

On November 25, 1975, the Soviet Union launched Cosmos 782, an unmanned spacecraft carrying biology and physics experiments from seven countries, including the Soviet Union and the United States. The Cosmos 782 mission marked the first time the Soviet Union had flown US experiments aboard one of her spacecraft. The flight, which lasted 19.5 days, differed from its predecessors, Cosmos 605 and 690, in the use of a large number of species (over 20) and the presence of an on-board centrifuge for artificial gravity. The US-involved experiments used rats, fruit flies, carrot tissue and cells, fish eggs, and radiation dosimeters. The Cosmos 782 experiments in general concentrated on comparing effects of low gravity versus artificial gravity on genetics, growth, development, and aging. The rat experiments focussed on low-gravity effects on endocrine, lymphoid, blood, muscle, and bone tissues. The spacecraft was recovered at 9:00 a.m., December 15, 1975, in Siberia. A field laboratory in the recovery area provided facilities for immediate postflight activities. Twelve of the 25 flight rats were autopsied in the field laboratory within 11 hours of recovery and tissue specimens taken; the remaining rats were saved for readaptation studies. Observations and tests were made on the flight rats and comparable ground control rats for 25 days postflight; at 25 days, the final examinations were performed, autopsies completed, and final tissue specimens taken. Rat tissues from the autopsies and the specimens from the other US-involved experiments were analyzed in US laboratories. An overview of the Cosmos 782 mission is presented, focussing on experiment hardware and preflight, on-orbit, and postflight activities relevant to the 11 US experiments on board the Soviet spacecraft.

INTRODUCTION

Cosmos 782 was the third in a series of Soviet Biological Satellite missions that began with Cosmos 605 in October, 1973 (Table 1). Cosmos 782 differed from its predecessors in three ways: the experiments used a large number of different species (over 20); an on-board centrifuge created artificial gravity for some specimens; and the Cosmos 782 mission was the first time the United States had ever been invited to participate in biological experiments on a Soviet spacecraft.

Seven countries participated in the Cosmos 782 mission with experiments in 15 experiment categories (Table 2). In addition to the US and the USSR, experiments were contributed by Czechoslovakia, France, Hungary, Poland, and Rumania. A total of 31 institutions in the 7 countries participated, with 12 of the institutions being from the United States. The Cosmos 782 experiments exemplified an underlying effort to maximize the scientific return from the mission; this effort was particularly reflected in the rat experiments, where 18 institutions from 5 of the 7 participating countries performed numerous biochemical and morphological studies on tissue specimens from virtually every system of the rat. Table 3 lists, by experiment, the participating institutions from each country.

The Cosmos 782 experiments, in general, concentrated on comparing the effects of weightlessness* versus artificial gravity on

*Although the term "weightlessness" is used here and in other reports of this volume, it is conceded and to be understood that complete weightlessness (absence of all accelerations) was not achieved. The spacecraft tumbled in orbit, imparting accelerations on the order of 1.7×10^{-7} to $1.5 \times 10^{-4}g$ to experiments at the edge of the spacecraft. Accelerations to materials more central received less. In any case, these accelerations are thought to be below biological threshold.

TABLE 1

SOVIET BIOLOGICAL SATELLITE MISSIONS

<u>MISSION PARAMETERS</u>	<u>COSMOS-605</u>	<u>COSMOS-690</u>	<u>COSMOS-782*</u>
LAUNCH DATE	31 OCT '73	22 OCT '74	25 NOV '75
RECOVERY DATE	22 NOV '73	12 NOV '74	15 DEC '75
MISSION LENGTH	22 DAYS	20.5 DAYS	19.5 DAYS
PERIOD OF REVOLUTION	90 MIN.	89.6 MIN.	90.5 MIN.
APOGEE	424 KM (261 MI)	389 KM (241 MI)	405 KM (251 MI)
PERIGEE	221 KM (135 MI)	223 KM (137 MI)	226 KM (140 MI)
ORBITAL INCLINATION	62.8°	62.8°	62.8°

*U.S. PARTICIPATION IN MISSION

TABLE 2

LIST OF EXPERIMENT CATEGORIES ABOARD COSMOS 782 AND THE SPONSORING COUNTRIES

<u>EXPERIMENTS WITH:</u>	<u>SPONSORING COUNTRIES:</u>
RATS	CZECHOSLOVAKIA HUNGARY POLAND U.S.A. U.S.S.R.
BACTERIA	U.S.S.R.
HIGHER PLANTS	U.S.S.R.
FUNGI	U.S.S.R.
MAMMAL CELL CULTURES	U.S.S.R.
HIGHER PLANT SEEDS	U.S.S.R.
TORTOISES	U.S.S.R.
DROSOPHILA	U.S.A. U.S.S.R.
CARROT TUMOR TISSUE	U.S.A. U.S.S.R.
GUPPIES	U.S.S.R.
CARROT EMBRYOID CELLS	U.S.A. U.S.S.R.
"BIOBLOK"	FRANCE RUMANIA U.S.S.R.
RADIATION DOSIMETRY	U.S.A. U.S.S.R.
ELECTROSTATIC PROTECTION	U.S.S.R.
FUNDULUS EGGS	U.S.A. U.S.S.R.

TABLE 3. LIST OF INSTITUTIONS PARTICIPATING IN THE COSMOS 782
MISSION BY EXPERIMENT CATEGORY

Rat Experiments

1. Institute of Biomedical Problems, Ministry of Public Health, USSR	USSR
2. Priorov Central Scientific Research Institute of Orthopedics and Traumatology, Ministry of Public Health, USSR	USSR
3. Central Scientific Research Institute of Stomatology, Ministry of Public Health, USSR	USSR
4. Leningrad Sanitary-Hygienic Institute, Ministry of Public Health, Russian SFSR	USSR
5. Institute of Gastroenterology	USSR
6. Institute of Medical Radiology, Academy of Sciences, USSR	USSR
7. Pavlov Institute of Physiology, Academy of Sciences, USSR	USSR
8. Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences, USSR	USSR
9. Bakh Institute of Biochemistry, Academy of Sciences, USSR	USSR
10. Institute of Experimental Endocrinology, Slovakian Academy of Sciences, Bratislava	Czechoslovakia USSR
11. Biology Department, Shafarik University, Kosice	Czechoslovakia USSR
12. Institute of Aviation Medicine	Poland
13. National Scientific Research Institute of Radiobiology and Radiohygiene, Budapest	Hungary
14. Ames Research Center, NASA, Moffett Field	USA
15. Stanford University	USA
16. University of Wisconsin	USA
17. Veteran's Administration Hospital, Syracuse	USA
18. Veteran's Administration Hospital, Seattle	USA

Table 3 - Continued

Bacteria Experiment

- | | | |
|----|---|------|
| 1. | Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
|----|---|------|

Fungi Experiment

- | | | |
|----|---|------|
| 1. | Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
|----|---|------|

Mammal Cell Culture Experiment

- | | | |
|----|---|------|
| 1. | Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
|----|---|------|

Higher Plant Experiment

- | | | |
|----|---|------|
| 1. | Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
|----|---|------|

Tortoise Experiment

- | | | |
|----|---|------|
| 1. | Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
|----|---|------|

Fruitfly Experiments

- | | | |
|----|---|------|
| 1. | Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
| 2. | Ames Research Center, NASA | USA |

Carrot Tumor Tissue Experiments

- | | | |
|----|---|------|
| 1. | Colorado State University | USA |
| 2. | Department of Physiology of Higher Plants, M. V. Lomonosov
Moscow State University | USSR |

Table 3 - Concluded

Carrot Embryoid Cell Experiments

- | | |
|---|------|
| 1. University of the State of New York at Stony Brook | USA |
| 2. Ames Research Center, NASA | USA |
| 3. Institute of Plant Physiology, Academy of Sciences, USSR | USSR |

"Bioblock" Experiments

- | | |
|--|---------|
| 1. Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
| 2. Institute of Nuclear Physics, Atomic Energy Committee | Rumania |
| 3. Laboratory of Biological Medicine, Department of Medicine,
University of Toulouse | France |
| 4. Laboratory of the Physics of Elementary Nuclear Particles,
Nuclear Research Center | France |

Radiation Dosimetry Experiments

- | | |
|--|------|
| 1. Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
| 2. University of San Francisco | USA |
| 3. Ames Research Center, NASA | USA |

Electrostatic Protection Experiments

- | | |
|--|------|
| 1. Institute of Biomedical Problems, Ministry of Public Health, USSR | USSR |
| 2. All-Union Scientific Research Institute "Electrostandart" | USSR |

Fundulus Egg Experiments

- | | |
|--|------|
| 1. L. Johnson Space Center | USA |
| 2. Louisville University | USA |
| 3. University of Texas | USA |
| 4. Bryn Mawr College | USA |
| 5. Embryology Faculty, M. V. Lomonosov Moscow State University | USSR |

genetics, growth, development, and aging. The rat experiments, having no artificial gravity aspect, focussed on the endocrine, lymphoid, blood, muscle and bone tissues. The US thrust involved 11 investigations using rats, fruit flies, carrot tissue slices, carrot embryoids, fish eggs, and radiation dosimeters. The US effort with rats and fruit flies examined the morphological and biochemical changes in animals exposed to weightlessness.

Non-Soviet scientist mission participation, in almost all cases, confined itself to activities outside the recovery area. The non-Soviet scientists either analyzed their recovered specimens in Soviet laboratories or analyzed them upon return to their respective countries. Soviet scientists, trained in the experiment-specific procedures, carried out the necessary recovery area activities and such on-site tasks as immediate autopsy and tissue preparation, all performed in the absence of the Principal Investigators. Those Soviet activities that supported and impacted the US experiments are discussed below; other activities are excluded. Along with the discussion of Soviet support activities, a description of mission operations, especially those pertinent to the US experiments, is offered to provide a foundation for understanding and interpreting the US experiment reports appearing here.

THE SPACECRAFT

The Cosmos 782 experiments flew in a modified Vostok spacecraft similar to that used for Cosmos 605 and 690, and similar to those used for the early Soviet manned spaceflights. The Vostok, a spherical craft about 8 feet in diameter (Figure 1), had a gross weight of approximately 5,000 lb, with a payload of around 2,000 lb. Batteries supplied most of the spacecraft power requirements during orbit.

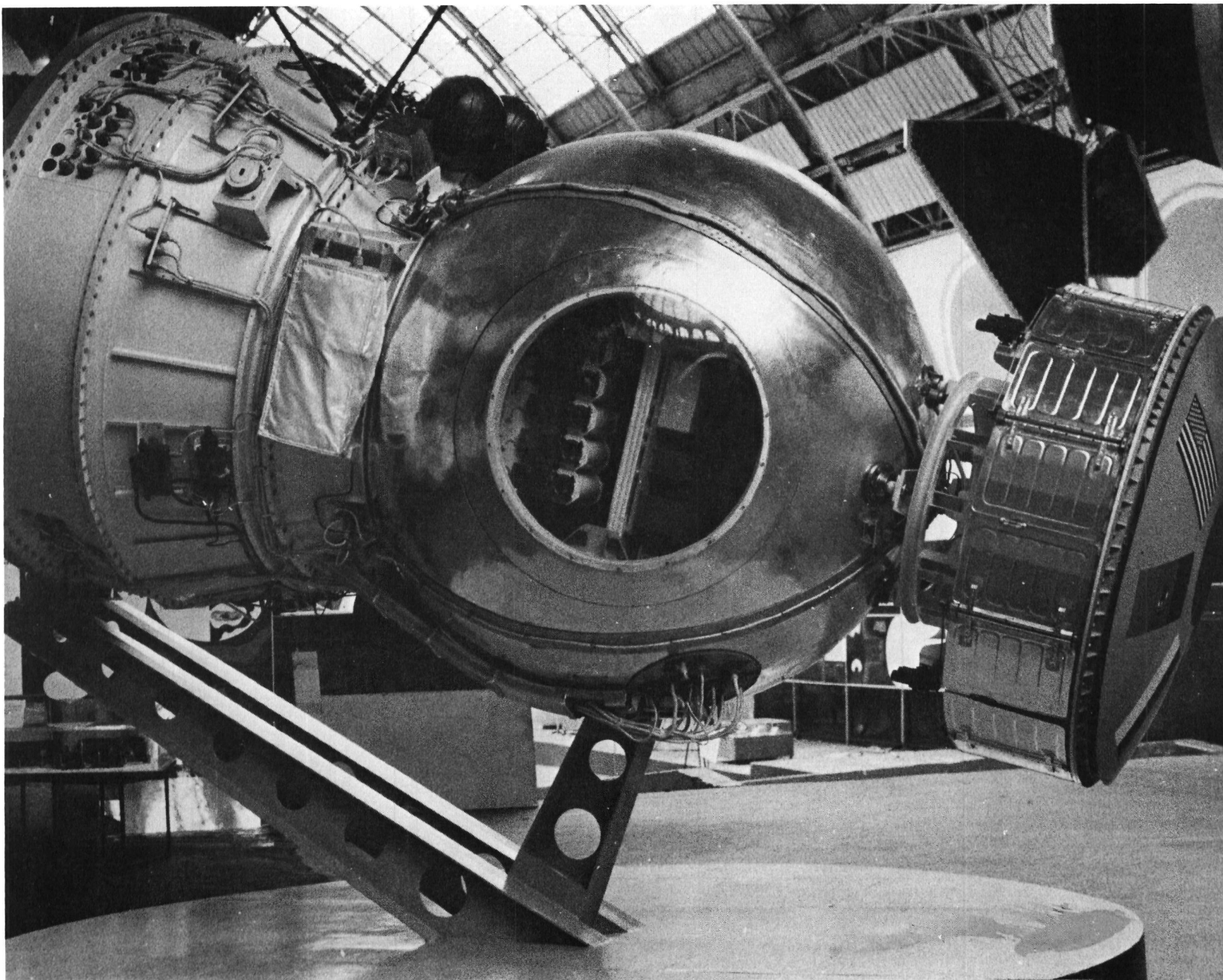


Figure 1 - Cosmos 782 Spacecraft on display in the Space Museum. Astakeno, USSR. A circular viewport was installed in the spherical craft when placed on display in the museum.

The spacecraft chamber (Figure 2) containing the biological materials maintained a controlled environment with a pO_2 between 150-210 mm Hg, a pCO_2 under 8 mm Hg, a total pressure between 740-870 mm Hg, a temperature between $19.2^{\circ} - 24.8^{\circ}C$, and a relative humidity between 40-63%. A ground-based mockup, the Synchronous Control chamber, duplicated the flight experiment conditions and contained duplicate experimental materials. During the flight, the Synchronous Control chamber maintained a controlled environment with a pO_2 between 145-205 mm Hg, a pCO_2 of less than 8 mm Hg, a temperature between $21^{\circ} - 24^{\circ}C$, and a relative humidity between 70-72%. Trace gaseous contaminants were not measured in the satellite cabin; however, the Synchronous Control chamber revealed a gradual increase in ammonia concentration throughout the flight period, but other toxic gas levels remained insignificant (Table 4).

The spacecraft interior contained three tiers for experimental materials (Figure 2): two platforms above and a space with attachment points below. The upper platform, a centrifuge 75 cm in diameter, rotated with a stabilized spin rate of 52 RPM. U.S. biological materials resided on the centrifuge in zones of 1 g and 0.6 g. Below the centrifuge platform lay a stationary platform, bearing experimental materials that duplicated those on the centrifuge. In addition to the two platforms, a third space below the stationary platform provided accommodation for 25 rats. The rats were in individual cages with five cages coupled to form a "block." Five blocks were joined and affixed to the attachment points in the space provided below the platforms.

EXPERIMENT HARDWARE

The US experiment specimen hardware comprised:

1. Specially machined acrylic canisters (Figure 3) contained the carrot slices for the Carrot Tumor Growth Experiment. Each cylindrical canister, about 8.5 cm in diameter and about 10.5 cm

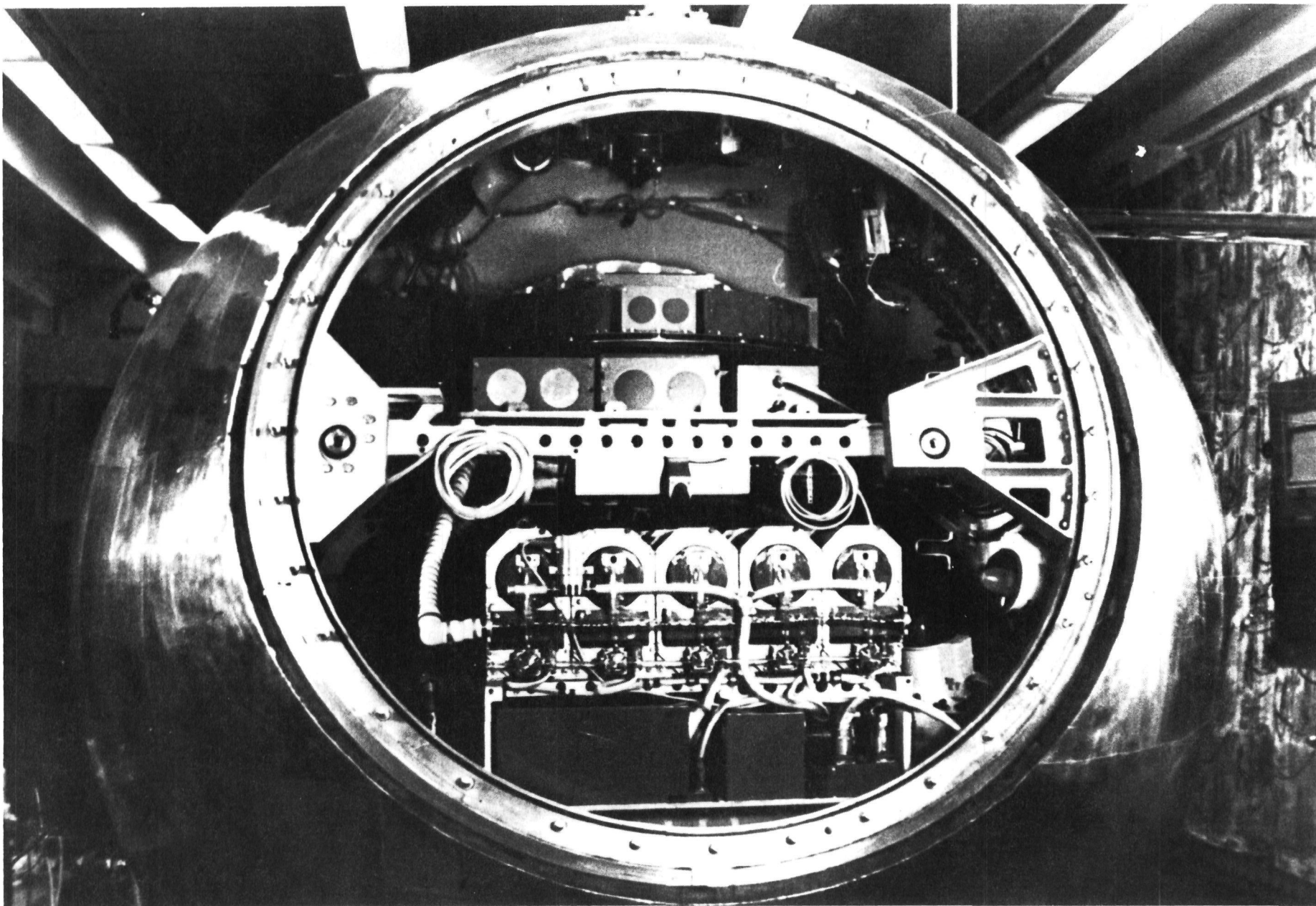


Figure 2. View of inside of cabin of the Cosmos 782 spacecraft. Below is an end view of a block of five rat cages; behind are four more blocks, giving a total of 25 cages. Just above the rat blocks, attached at both sides of the viewport, is the stationary platform with three flight containers visible. Directly above these stationary flight containers is the centrifuge with about seven flight containers visible.

TABLE 4
SEVERAL BASIC VOLATILE METABOLITES IN SYNCHRONOUS
CONTROL CABIN OF COSMOS 782 MISSION

Components determined	Length of experiment (days)																		
	Back-ground	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. Ammonia (mg/m ³)	0.1	0.4	0.25	0.8	0.8	0.8	0.9	0.95	1.0	1.3	2.6	5.3	6.1	6.0	7.8	11.0	14.0	16.2	18.3
2. Acetone (mg/m ³)	0	0	0	-	-	0	-	-	0	-	-	0	-	0.2	-	0.3	0.2	0.2	0.3
3. Aldehydes (according to formal- dehyde) (mg/m ³)	0	0	0	-	-	0	-	-	0	-	-	0	-	0	-	0.1	0.2	0.1	0.2
4. Organic acids (mg/m ³)	0	0	0	-	-	0	-	-	-	-	-	-	0	-	-	-	-	-	0
5. Oxidiz- able compounds (mg O ₂ /m ³)	30	-	60	-	96	-	96	-	108	-	108	-	108	-	120	-	108	-	-

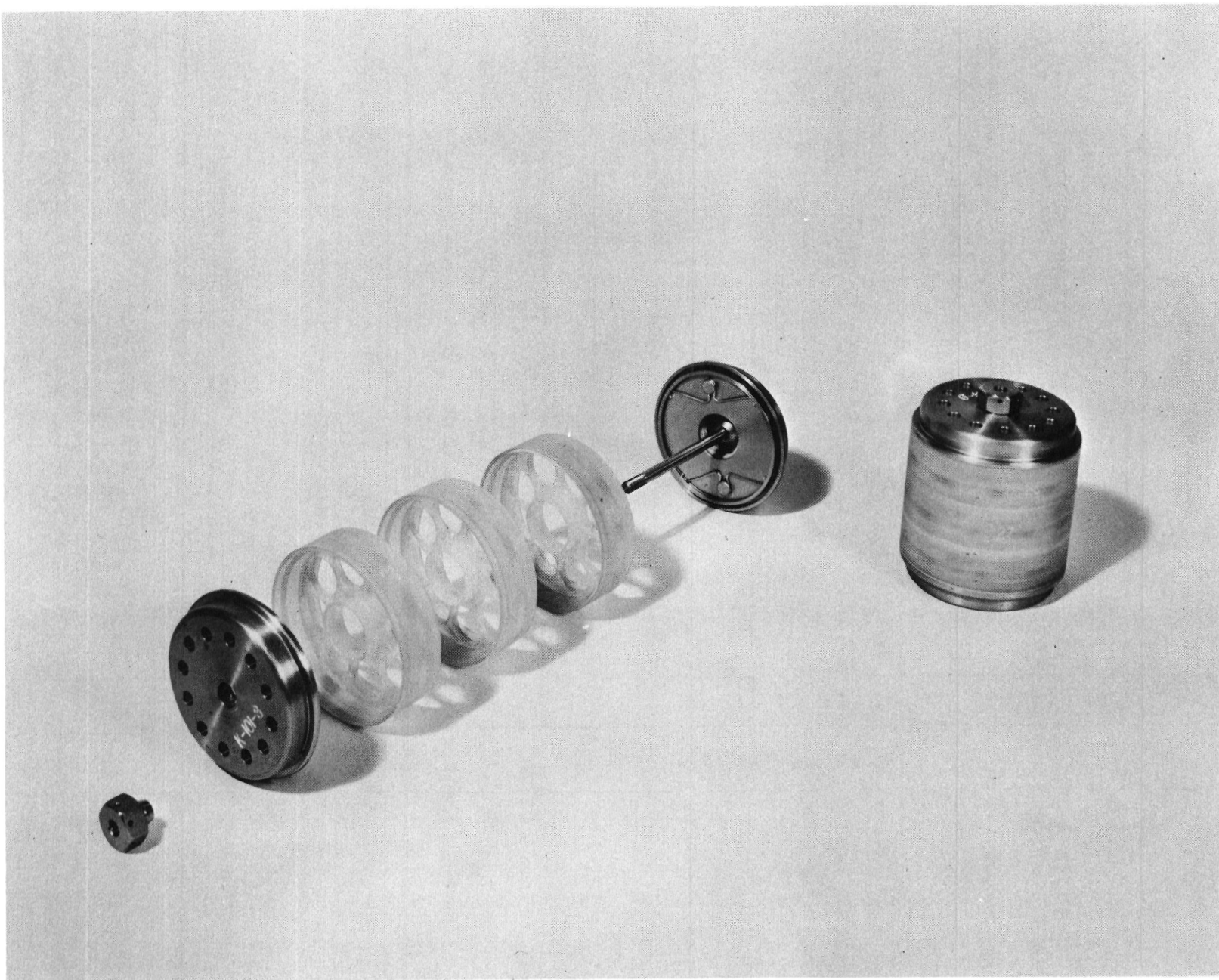


Figure 3. Acrylic canisters for the Carrot Tumor Growth Experiment.

in overall height, consisted of a stack of three closely fitted dishes, each dish being about 2.3 cm deep. A rimmed center hole, about 2.5 cm inside diameter, penetrated the floor of the dish. The center hole allowed passage of a threaded metal rod that helped clamp together a complete canister of stacked dishes. Evenly spaced around the center hole, four smaller holes, about 2 cm in diameter, pierced the floor of the dish. These four smaller holes provided seats to accommodate the uniformly cut and sized, press-fitted disks of carrot tissue. Two machined, anodized aluminum caps clamped and sealed three stacked dishes by means of one threaded rod and one nut into one complete canister which could contain up to 12 carrot slices. Each cap contained a filter pad and 12 holes to permit air passage into the container. Figure 4 shows two canisters, packing material, and the flight container.

2. Specially constructed canisters (Figure 5) housed plastic petri dishes for the Carrot Embryoid Culture Experiment. The stacked, 5-cm-diameter, sealed petri dishes contained clones of carrot cells in a thin layer of agar medium. Each cylindrical canister, about 6.5 cm in diameter and about 10.5 cm in height, consisted of an acrylic tube with the ends threaded to accommodate two anodized aluminum alloy end-caps. Each end-cap, about 6.7 cm outside diameter, had a circle of twelve 0.6-cm-diameter holes for air entry. Standoffs made of 0.063-inch-thick "microsil," a rubbery plastic, cushioned and secured the petri dishes within the acrylic canister tube. The four standoffs were simply four equally spaced legs extending from a circle, about 5 cm in diameter, all cut from a single flat piece of microsil. In loading the petri dish stack, the microsil circle flattened against the top of the stack and the four legs folded down the sides. The microsil cushioned the stack against one canister end-cap, and pyrell foam of number 4 density cushioned and secured the stack against the other end-cap. Each cap contained one glass fiber pad, about 25 mils thick, to filter entering air. Manual tightening secured the end-caps to the

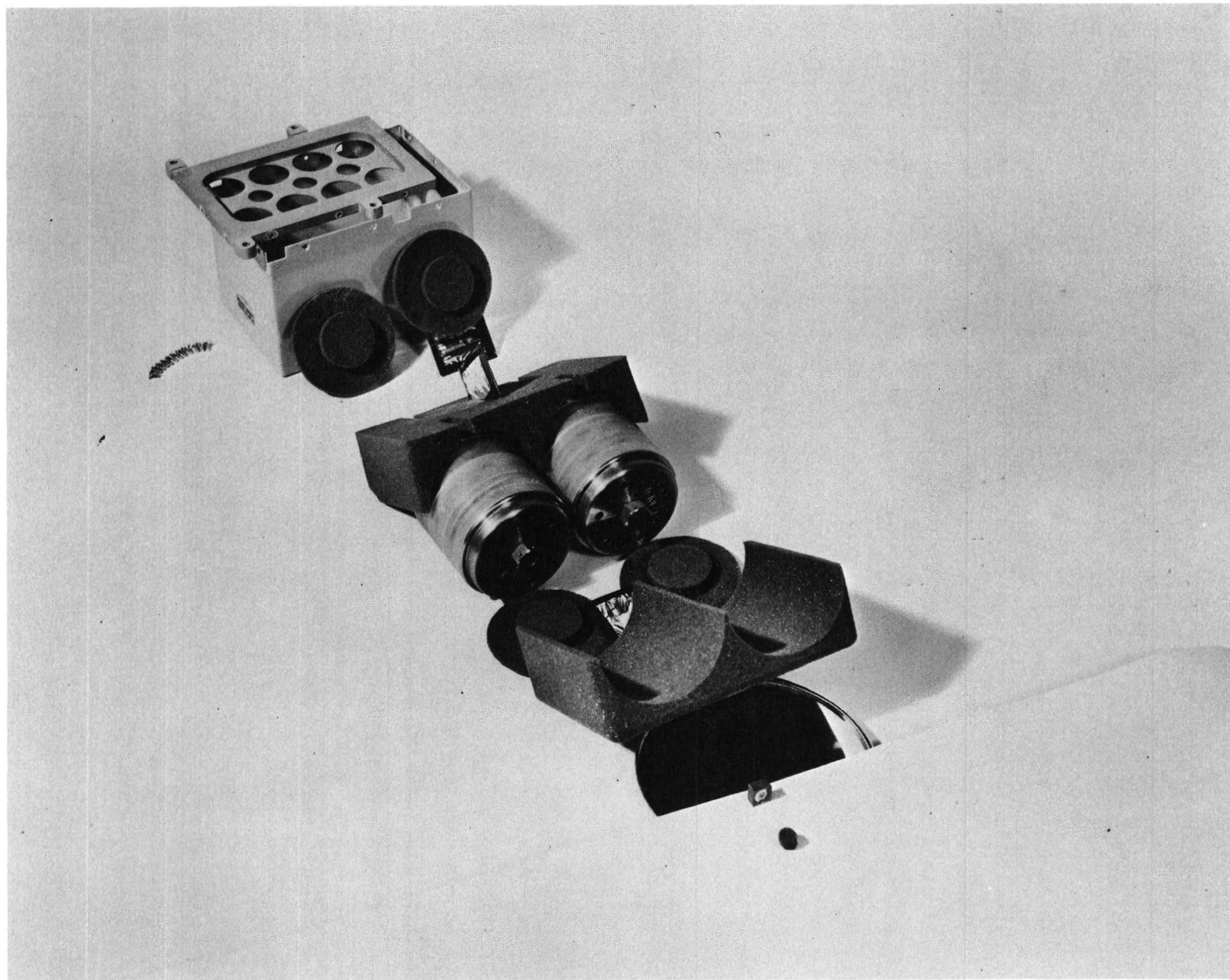
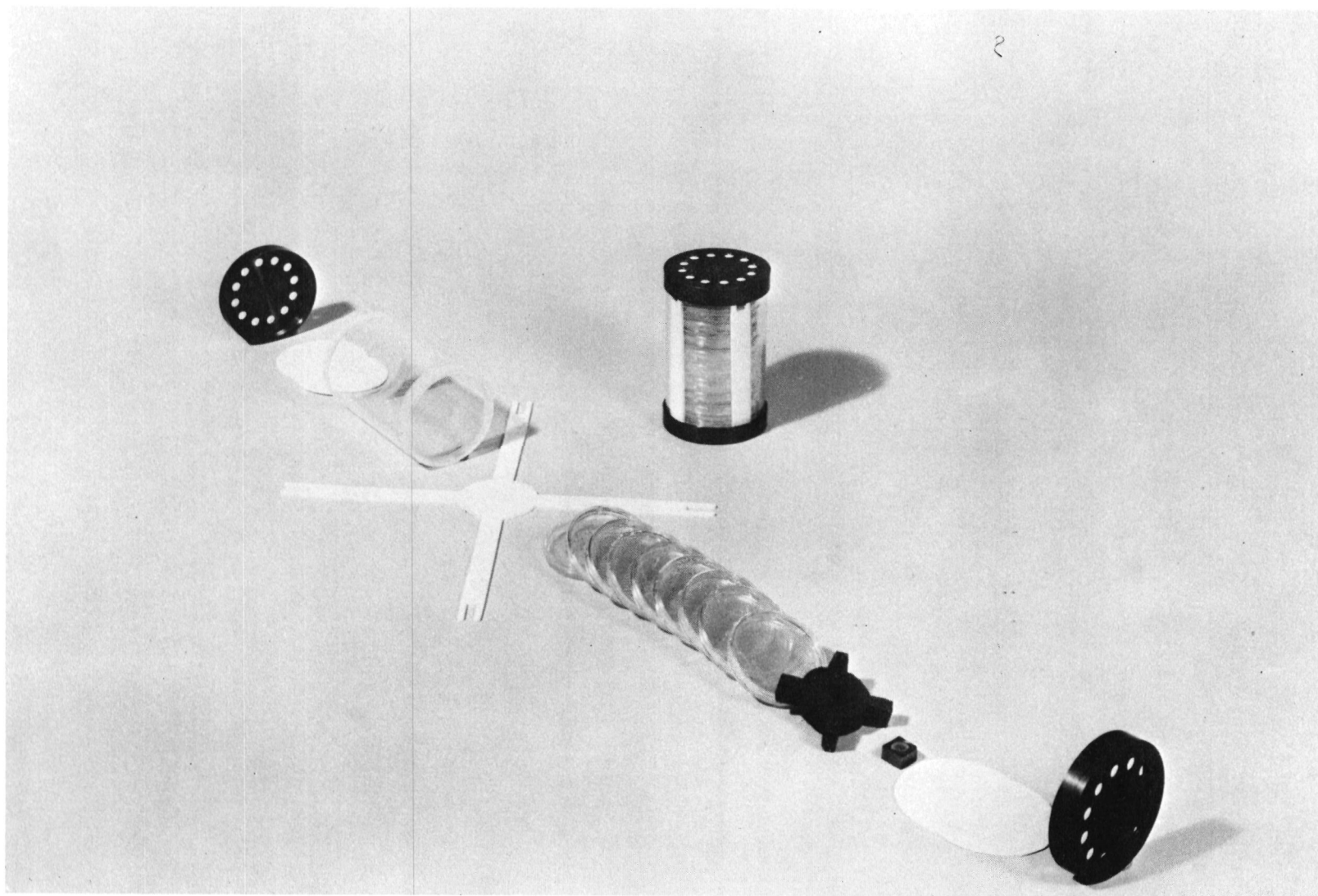


Figure 4. Canisters for Carrot Tumor Growth Experiment, packing foam, and (at the rear) the flight container.



17

acrylic tube. A complete canister consisted of one acrylic tube, two end-caps, two filters, one max-min thermometer, one pyrell foam pad, nine petri dishes, and one four-legged standoff cushion. Figure 6 shows the canister (foreground), packing foam (midground), and the flight container (background). The cuboid box to the left of the packing foam houses another experiment, the Fundulus Egg Experiment (discussed below), which shares the same flight container.

3. Radiation detectors were fabricated for the HZE Dosimetry Experiment. Cellulose nitrate and Lexan polycarbonate films, 100 and 250 μm thick, respectively, provided the building materials for the two detector designs: thin and thick designs. The minimum LET value detectible with these plastics was $\sim 100 \text{ KeV}/\mu\text{m}$ • Tissue. The thin detector package was 5 cm square and 0.15 cm thick. Aluminized mylar wrapping protected the films from ultraviolet exposure. The thin detector, consisting of a stack of seven thin plastic films held with tape, measured high-LET particle flux and integral LET spectrum. The thick detector package (9 cm square and 2 cm thick), consisting of a stack of 75 plastic films, measured the charge spectrum. Cosmos 782 flew a total of 12 thin detectors in sets of three mutually orthogonally placed packages. One set each was affixed to the flight containers of the K102/K104 and K101 experiments on the centrifuge and on the stationary platform. Two thick detectors were placed on the stationary platform.

4. A machined aluminum, two-chamber, cuboid case, 8.9 x 9.5 x 9.8 cm outside dimensions, carried the Fundulus Egg Experiment (Figure 7). Each chamber held five flattened polyethylene bags, 7.0 x 8.4 cm inside dimensions, and separators made of thin, perforated sheets of pyrell foam. The bags contained the biological material suspended in 21 ml of sterile, filtered, 21-parts/thousand Instant Ocean. A perforated plate, held in place by four Allen screws, closed the case. The Soviets supplied the flight container, a sheetmetal box screwed to a

ORIGINAL PAGE
BLACK AND WHITE PHOTOGRAPH

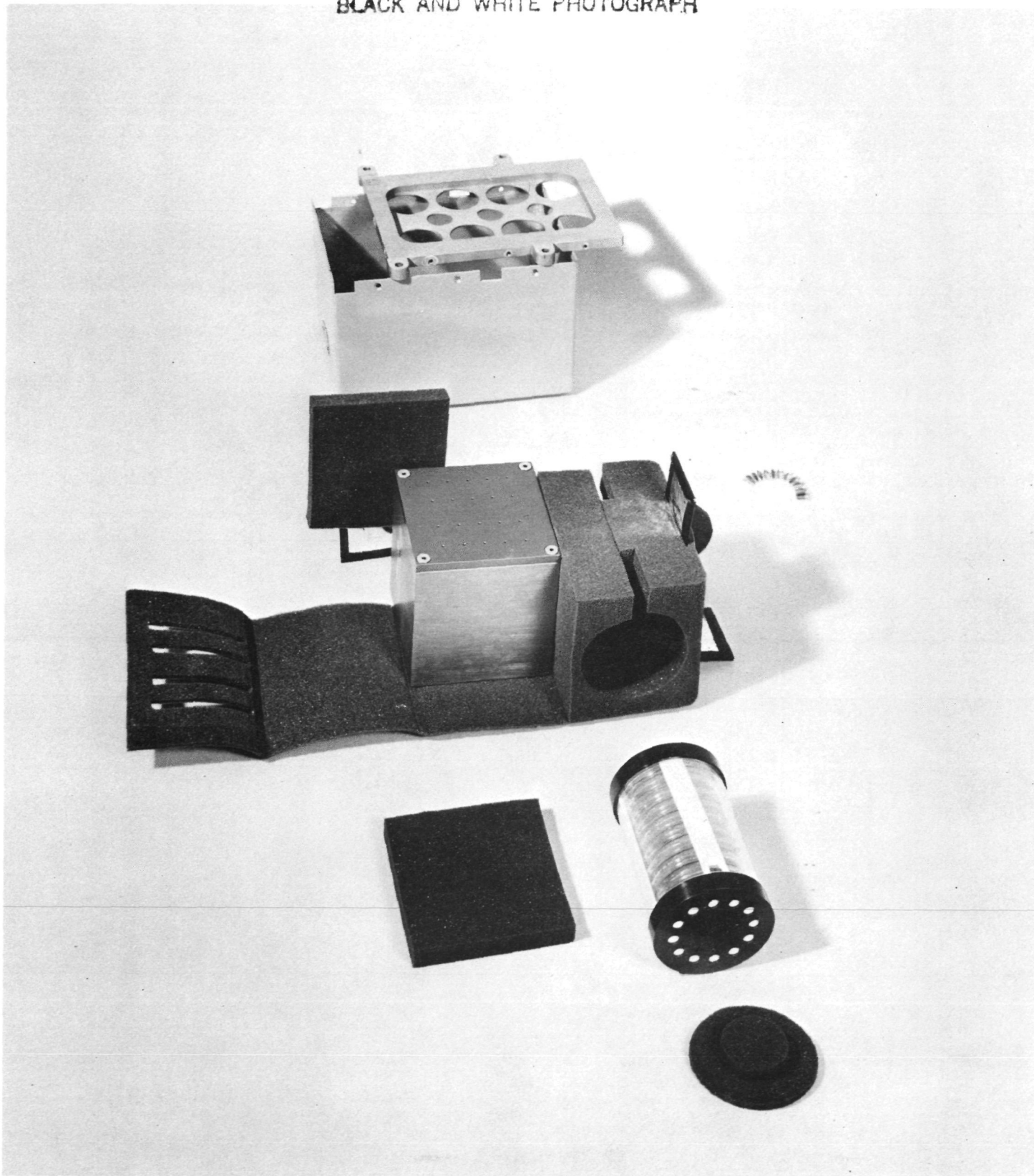


Figure 6. Fundulus Egg Experiment and the Carrot Embryoid Culture Experiment sharing a flight container. The cuboid to the left of the foam cushion is the egg container and the acrylic canister in front houses the embryoid cultures. The box in the rear is the flight container.

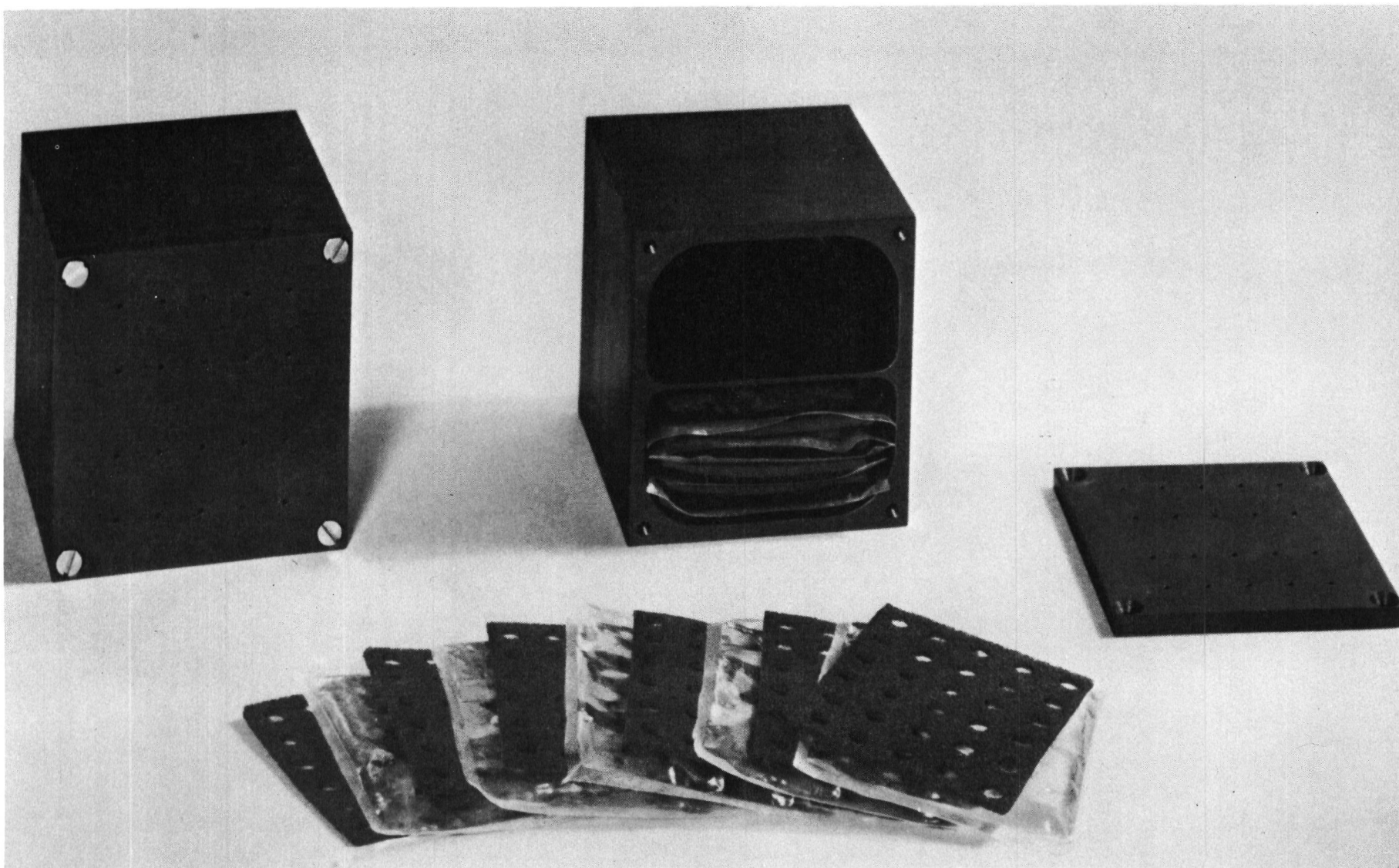


Figure 7. Container for the Fundulus Egg Experiment. The eggs were suspended in Instant Ocean in flattened polyethylene bags and stacked with separators into the container.

machined base, which, in turn, had screw holes to match spacecraft attachment points. A US-furnished, low-temperature transporter was used to provide an environmental temperature of $10^{\circ} \pm 0.2^{\circ}\text{C}$ during transit. This experiment shared the flight container with another US experiment, the Carrot Tumor Growth Experiment (Figure 6).

5. Soviet-furnished, individual cages housed the specimens for the Rat Experiment. Each cage (Figure 8) contained its own light, food, water, air circulation, and waste management systems. A single cage consisted of two cylinders that intercommunicated along their lengths, one above the other. The upper cylinder, about 9.5 cm in diameter and about 20.8 cm long, housed one rat; the lower cylinder, similarly dimensioned, contained the waste collection trap and provided the exhaust avenue for exiting air. Cabin air entered a port, followed a duct above the rat chamber, entered the chamber through ceiling holes, dispersed downward over the animal, flushed through the floor grill, filtered through the waste trap, and exhausted from the lower cage chamber; the trap rotated to present a clean surface at two-day intervals. After exiting the cage, waste trap air passed through activated charcoal filters and returned to the cabin. The rear wall of the animal chamber bore lights, which provided a 12/12 hour light/dark cycle with a 2-lux intensity; an automatic watering device provided water ad libitum, and an automatic feeder cup dispensed 10 gm of paste diet (Table 5) at 6 hour intervals. The front wall contained an entrance door above and the waste trap rotation mechanism along with a signal conditioner housed in a box below. The animal chamber had a flexible plastic liner surrounded by wire coil transducers that sensed gross animal activity.

6. The Soviets provided sheetmetal flight containers that housed standard, cotton-stoppered fruit fly vials with standard food medium for the Fruit Fly Experiment.

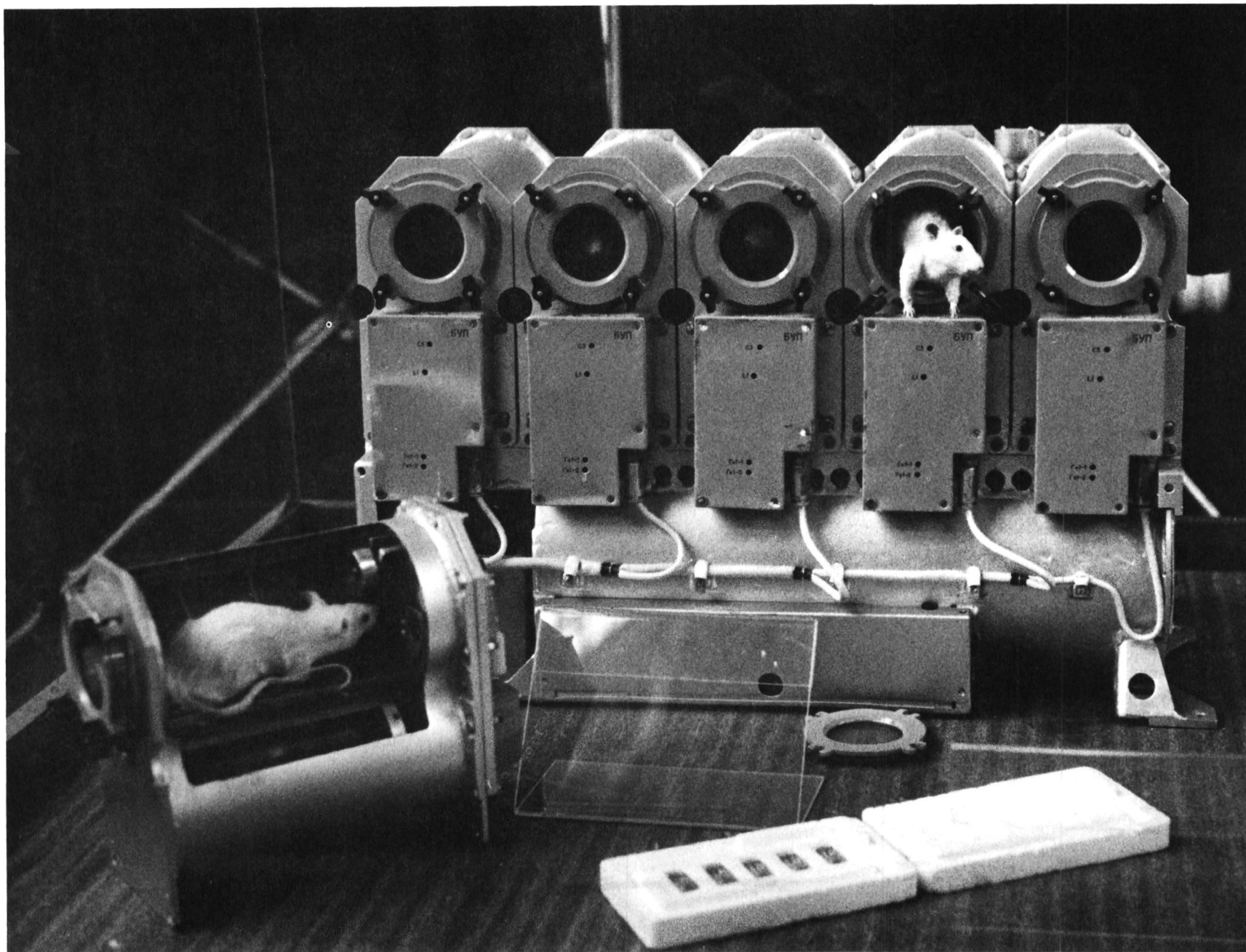


Figure 8. Behind is a block of five rat cages which shared common water and food reservoirs. In front is a view of a single cage showing the internal structure.

Table 5 Composition of the Paste Diet Fed to Rats During the Cosmos 782 Mission

Constituents	Quantity, grams	Minerals	Quantity, mg/40 g of diet	Vitamins	Quantity, μ g/40 g of diet
Casein	3.0	Sodium (Na ⁺) Chlorine (Cl ⁻)	82.6 20.4	B ₁	64.8
Corn starch	3.0	Potassium (K ⁺) Phosphorus (P ^{+3,5})	84.9 113.4	B ₂	62.4
Sucrose	6.7	Calcium (Ca ⁺⁺) Iron (Fe ⁺⁺)	122.2 2.1	B ₃	240
Sunflower seed oil	1.7	Iodine (I ⁻) Zinc (Zn ⁺⁺) Copper (Cu ⁺⁺)	0.01 0.1 0.1	B ₆ B ₁₂	50.5 1.5
Dry brewers yeast	1.0	Cobalt (Co ⁺⁺) Flourine (F ⁻) Aluminum (Al ⁺⁺⁺)	0.02 0.002 0.01	E A	1380 20
Salt mixture	0.6	Magnesium (Mg ⁺⁺) Sulfur (S ⁺⁶)	9.3 14.2	D K	6 25
Water	24.0	Manganese (Mn ⁺⁺)	0.9	Biotin Folic acid Choline p-amino Ben- zoic Acid Nicotinic acid	5.0 5.0 ? ? 493.6

PAYLOAD

The Cosmos 782 US-involved payload comprised:

1. Carrot Tumor Growth Experiment. Crown gall tumors developed on carrot disks resided on the centrifuge and on the stationary platform. Analysis of various biological substances from the disks provided data for accomplishment of the main experimental objective: to assess the effects of weightlessness and weightlessness versus artificial gravity and versus the null-gravity environment of a ground-based clinostat. The substances for which the disks were analyzed included amylose, sucrose, glucose, peroxydase, and glutamine synthetase; overall tumor growth, however, provided the most important parameter in the study, for growth, being a measure of overall metabolic activity, provided data directly related to the main experimental objective.

2. Carrot Tissue Culture. This experiment used cultured carrot totipotent cells, which have the power to develop and to differentiate as do zygotic embryos. Consequently, these cells can initiate complex growing regions such as shoots and roots. This experiment was aimed at answering the question of whether cells of higher plants, such as the carrot, could develop normally in the weightlessness of space, emulating their known ability on Earth to multiply, develop, and form organs, embryoids, and normal plantlets.

3. HZE-Particle Dosimetry. Twelve thin and two thick radiation detectors were attached to flight containers and to the stationary platform. These plastic film detectors yielded data that were necessary in assessing the potential hazard of cosmic radiation during prolonged spaceflight.

4. Fundulus Egg Experiment. Eggs of the fish Fundulus heteroclitus were carried in synthetic sea-water-filled plastic

bags. This experiment was an outgrowth and continuation of prior Apollo-Soyuz tests to explore possible harmful effects of weightlessness on the development and growth of biological systems. This experiment advanced prior ones in the use of the on-board centrifuge as a 1-g control treatment.

5. Rat Experiment. The US involvement with rats studied the effects of weightlessness with several technical approaches.

Immune response: this study examined the effects of spaceflight on cell mediated immunity.

Erythrocyte survival: flight animals showed an altered erythrocyte potential life span and random hemolysis.

Hormone responses: observations indicate some effects of spaceflight on endocrine function which point to the adrenal and pituitary glands.

Histology studies (several): examination of stomach tissue provided evidence on whether stress of spaceflight produced gastric ulceration, erosion of the gastric mucosa, or other histologic changes; lymph node tissue of flight animals showed alterations in lymphocyte and pyknotic and necrotic cells as well as debris; light and electron microscopic examinations of rat retina provided data on cosmic particle damage; and flight animals showed altered bone formation, resorption, and mineralization.

6. Fruit Fly Experiment. Specimens of Drosophila melanogaster developed and spent the first days of their adult life in space. After the 19.5-day exposure to weightlessness, the flies yielded morphological, chemical, and behavioral data that was used to evaluate any effects attributable to weightlessness.

MISSION OPERATIONS

Two kinds of Ground Control experiments supported the Flight experiments aboard the spacecraft - the Synchronous Control and the Vivarium Control. The Synchronous Control, a spacecraft

mockup, duplicated, as closely as possible, the environment of the spacecraft during flight. The spacecraft mockup also contained duplicates of all experiments that flew in the actual spacecraft with the purpose of differentiating effects of weightlessness from the other effects of flight. Both the Control specimens and Flight specimens had the same hardware, food, water, lighting, temperature, and quality and flow of air. The mockup centrifuge rotated at the same rate as the flight centrifuge, but being on the ground, the centrifuge administered a greater resultant centrifugal force to the specimens than the centrifuge in orbit.

Before the start of the Synchronous Control, the rats received simulated launch stresses. For 10 minutes, the rats received 110-db noise level and a vibration frequency of 50-70 Hz at an amplitude of 0.04 cm; immediately following the noise and vibration stresses, the animals received a 10-minute acceleration with a plateau of 4 g for 7 minutes. Following the launch stresses, the rats were loaded into the Synchronous Control mockup cabin. The other US experiments received the same launch stress profile and were loaded into the Synchronous Control hardware on December 1, 1975. The centrifuge was started on December 1, 1975, and the mockup sealed on December 2, 1975. After a simulated flight period, the centrifuge was stopped on December 21, 1975. The Synchronous Control was unloaded and the rats subjected to reentry stresses on December 20, 1975. The other US experiments were subjected to reentry stresses on December 21, 1975. The reentry stresses comprised: an acceleration for 5 minutes with a plateau of 6 g for 3 minutes, followed by an impact shock of 50 g with a duration of 10 msec. Afterward, all control materials were handled exactly as the Flight specimens.

The Vivarium Control Experiment, running parallel with the Flight experiment, provided data on minimally stressed specimens for

comparison with the Flight and Synchronous Control groups. The Vivarium maintained a temperature of $21^{\circ} \pm 2^{\circ}\text{C}$ with a relative humidity of $80 \pm 5\%$. Standard polyvinyl cages, 45.0 x 31.0 x 16.0 cm, housed 3-4 rats each throughout the flight and postflight periods. The rats received the same paste diet, 40 g per animal per day (Table 5) as the Flight and Synchronous Control animals during preflight and orbit. During postflight, the Vivarium rats and the recovery rats returned from the other two groups received 45 gm of paste diet per rat per day.

Prelaunch Activities

Through the month of November 1975, experimental materials arrived in Moscow from the laboratories of the Principal Investigators. The Institute of Endocrinology of the Slovakian Academy of Sciences made available 200 pathogen-free Wistar rats from which to select the Flight and Control animals. At launch and the start of the control experiments, the animals weighed about 212 gm and were 63 days old. The Soviets supplied specimens of a Domodedovo-32 strain of fruit fly, Drosophila melanogaster. Other US experimenters with other materials supplied their own specimens and materials. Preparations for starting the experiments included delivery of all materials and all specimens for the three parallel experimental groups: the Flight, Synchronous Control, and Vivarium Control experiments. In addition to overall preparations, the rat studies required that certain identical treatments be performed on the rats of all three experimental groups before the initiation of the experiments. The treatments involved injections, changes in diet, and cage training. Since the time lines for the treatments were comparable in the three groups, the following schedule is given for the Flight experiment group only and is an outline of the treatments and their occurrences in days before the initiation of the experiment (at launch for the Flight group):
¹⁴C glycine injection at 15.5 days, start paste diet at 14 days, cage training at 11 days, Listeria vaccine injection at 6 days,

and Declomycin injection at 3 days (Table 6). During prelaunch activities, the specimens were housed in the Vivarium.

On-Orbit Activities

The Soviets launched the Cosmos 782 Biological Satellite from Plestsk, USSR, at 8:00 pm, on November 25, 1975. Cosmos 782 achieved a 219 n. mi. (405 km) apogee by 122 n. mi. (226 km) perigee orbit at an inclination of 62.8°. The Vivarium Control began with the launch; approximately five days later, the Synchronous Control began. On-board sensors provided housekeeping and biological data. Cosmos 782 telemetry carried to Earth information on rat motor activity and body temperature.

Although memory blocks occasionally overloaded, it was clear that rat nocturnal activity exceeded the diurnal activity in both Flight and Control rats. The Flight rats, nevertheless, showed slightly greater nocturnal activity than the Control animals.

Five rats received body temperature telemetry implants. The short range transmitters sent body temperature data to receivers in the spacecraft cabin. The Cosmos 782 recording/telemetry system then periodically transmitted the body temperature to Earth. The Flight animals on the average had a lower body temperature than the Synchronous Control animals, although both groups showed hypothermia. It was thought that airflow, isolation, and confinement significantly contributed to the lowered body temperature in the two groups. Preflight tests showed that body temperature does not fall significantly when the cabin temperature is sufficiently high. During the flight, the cabin temperature was not at the higher preflight test level. It was thought, nevertheless, that the heat regulation system of the organism would be involved in a reaction to weightlessness primarily by way of altered muscular activity.

TABLE 6

CHRONOLOGY OF MAJOR MISSION OPERATIONS
IN RAT EXPERIMENT FOR COSMOS 782

Activity	GROUPS OF ANIMALS		
	flight	synchronous	vivarium
Delivery of animals to Moscow and start of clinical-physiological tests	November 4, 1975	November 4, 1975	November 4, 1975
Implantation of body temperature transmitters	November 6, 1975	November 6, 1975	November 6, 1975
Injectons:			
- glycine C-14	November 10, 1975	November 15, 1975	November 10, 1975
- Listeria	November 19, 1975	November 25, 1975	November 25, 1975
- Declomycin (first injection)	November 22, 1975	November 27, 1975	November 25, 1975
- Declomycin (second injection)	December 18, 1975	December 23, 1975	December 18, 1975
First killing	December 15, 1975	December 20, 1975	December 21, 1975
Second killing	January 10, 1976	January 15, 1976	January 12, 1976

Postflight Activities

Recovery occurred at 9 am, December 15, 1975, in Siberia; the flight lasted 19.5 days. Owing to the requirements of handling biological material within 6 hours of landing, a recovery team of engineers and scientists was airlifted to the recovery site from a nearby air base during the orbital flight. The team set up a field laboratory which comprised three tents of rubber-coated, heat-insulated cloth. A forced air system maintained an interior temperature of $+18^{\circ}\text{C}$ to $+22^{\circ}\text{C}$. Two gasoline-electric units provided the energy for the laboratory.

Rat autopsies and tissue preparations done in this field laboratory provided data and tissue specimens for further studies in Soviet and non-Soviet laboratories. Gross examination of the recovered rats revealed a generally satisfactory condition: the rats had clean hair and tails in most cases with no bald areas, while visible mucous membranes and bare skin areas were pale pink, clean, and undamaged. Flight rats gained an average of 43 g during the flight, while the Synchronous Control rats gained an average of 70 g. The lag in weight gain in the Flight rats was attributable to physiological reactions to the generally more stressful environment during the flight. Autopsies, within 11 hours of recovery, revealed no pathological disturbances attributable to spaceflight. The following changes were found: increase in weight of the adrenals; reduced weight of thymus, spleen, and certain hindlimb muscles; and a tendency toward reduced weight of lymph nodes.

The readaptation studies involved observations and tests of the rats for 25 days postflight when, at this time, the rats from all three experimental groups were autopsied. During the first few days of readaptation, the Flight rats displayed listlessness and low motor activity. They hobbled when they walked and often lost their equilibrium when they stood. When reaching to examine some highly placed object, they did not stand on their toes, but used

the entire foot for support. Static endurance showed altered ability in Flight rats. The evaluation device consisted of rods coiled vertically down from a platform fixed at a height of 180-200 cm. The maximum time a rat could remain on the rods indicated relative endurance. Before the flight, all experimental groups showed no difference. At 6 hours postflight, the Flight and Synchronous groups showed a similar reduction in endurance, about three times. Although the amount of reduction appeared similar in both groups, the Flight rats took much longer to achieve recovery than the Ground Controls. Although it appears that the confined housing conditions determined the lowered endurance, the spaceflight environment, weightlessness, had an effect, as clearly evidenced by the protracted recovery of the Flight rats. Vestibular function also seemed to be altered by spaceflight. In tests where animals were photographed as they fell from a pole, it was discovered that many of the Flight animals made no attempt to turn in the air to land feet first. Those animals who attempted to turn did not complete the rotation and, as a rule, landed tail first. Another vestibulo-motor test, a balance test, also indicated a vestibular function disturbance. Flight rats required a significantly longer time than Control rats to find a balance position on an unstable beam. The vestibulo-motor disturbances disappeared by the tenth day of readaptation. The Synchronous Control and Vivarium Control animals remained practically unchanged in equilibrium function for the whole readaptation period. By the 25th day, all test rats had caught up to normal weights, and the majority of all test parameters of vital activity had returned to normal.

Analysis of these results and others clearly indicate that there are evidences of stress which are attributable to prolonged weightlessness and not to other dynamic factors of the flight. The experiments with rats on Cosmos 782 generally supplemented existing information on the mechanisms of adaptation by living systems to prolonged weightlessness.

With the completion of the postflight testing and the final examinations and autopsies between January 10 and 15, 1976, the 25-day postflight readaptation study came to a conclusion and, with it, mission operations for Cosmos 782. But for many investigators who participated in the mission, it was the beginning of the task of processing specimens, evaluating data, and reporting on the results. The reports in this volume are the results of the 11 US studies that were aboard the Soviet Cosmos 782 spacecraft.

ACKNOWLEDGMENTS

A special thanks is owed to the many persons who contributed to the success of this first United States-Soviet joint effort in life science flight experiments. Special acknowledgment is extended to Lawrence P. Chambers and Richard C. Simmonds, whose able managerial efforts facilitated and helped make possible the US part in the Cosmos 782 co-venture. Acknowledgment is also cordially extended to David L. Winter, Harold P. Klein, and Joseph C. Sharp for their managerial support.

RESPONSE OF CROWN GALL TISSUE TO THE SPACE ENVIRONMENT: RESIDUAL
CARBOHYDRATES IN SUPPORTING TISSUE

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ABSTRACT

Amounts of amylose, sucrose and glucose in the gall supporting flight discs and for comparable control discs were determined in an effort to detect the effect of weightlessness on the levels of those carbohydrates as a possible measure of respiration. Both amylose and sucrose decreased in concentration while in the flight environment; however, there was a marked increase in the concentration of glucose. There was no detectable difference between the tissue subjected to weightless environment compared to those subjected to the g environment.

We have concluded that the overriding environmental parameter was water stress which induced the tissue to increase the number of small molecules so that water could be retained by the tissue.

INTRODUCTION

Dedolph et al. (1) have shown that plant tissue which is gravity compensated on a clinostat has a greater rate of respiration than similar tissue exposed to a normal gravitational environment. We, therefore, decided that it would be wise to determine whether this response occurred in the weightless situation of the Soviet Biosatellite.

Since it would not be possible to study gas exchange under such conditions, we decided to study the depletion of carbohydrate reserve during the flight. These preliminary results do not support the hypothesis that respiration is more rapid under weightless conditions than in the 1 g environment.

METHODS AND MATERIALS

During the preparation of the flight material, which is described elsewhere, a piece of carrot tissue from each carrot, which was as much like the flight material as possible, was retained in a frozen condition until it was analyzed.

Upon return of the flight material, the galls, in a frozen condition, were removed for other studies and the remaining frozen discs along with the control non-flight discs were lyophilized. Each dried disc was macerated using a mortar and pestle.

From each disc sample, two samples (approx. 50 mg) of known size were suspended in 3 ml of 80% ethanol. These suspended samples were then centrifuged at 1200 G for 15 min. The supernatant was removed and the pellet was resuspended in 80% ethanol and subjected to centrifugation again. The supernatant was combined with the supernatant from the first centrifugation, and brought up to 10 ml with 80% ETOH.

Each pellet was dried then suspended in 5 ml of 0.01 M acetate buffer, pH 4.6. Each sample was incubated for 4 hr at 35° with 40 mg amyloglucosidase (activity 3000 units/mg, Sigma). The samples were constantly mixed with a magnetic stirrer. Each sample was then centrifuged as before. A small aliquot of the pellet was tested with IKI for residual starch. No starch was detected at this stage of sample preparation.

The pH of these samples of glucose from amylose was then adjusted to pH 7.0 with phosphate buffer. Aliquots of the combined supernatant were analyzed for glucose using the glucose oxidase system of Worthington Biochemical Corporation. The supplier's recommended procedure was followed except that no HCl was added to stop the reaction but rather the reaction was allowed to go to completion (1 hr at 35°C). This procedure has been previously reported (2).

The supernatant from the original centrifugation was used for glucose and sucrose analyses. It is admitted that this does not account for all of the soluble carbohydrates, but it should be representative. Aliquots of 0.1 ml were taken from each alcoholic solution and air dried.

For sucrose analysis, the samples were dissolved in 0.01 M acetate buffer at pH 4.6. To this, 0.1 ml of the buffer solution was added containing 1 mg invertase/ml (200 units/mg) which was supplied by Sigma. This mixture was incubated at 35° for 1 hr. The pH was then adjusted to 7.0 with 5% sodium bicarbonate and glucose was analyzed as previously described. For total sucrose, the values for the glucose analysis

described below were subtracted to obtain actual sucrose levels.

For glucose analysis, dried supernatant aliquots were dissolved in 0.1 M phosphate buffer at pH 7.0. The samples were analyzed for glucose as previously described.

RESULTS AND DISCUSSION

It should be pointed out that the study of carbohydrates was initiated late in this program as an effort to study differences in respiration and to use all material that was flown. Because of the time between the introduction of these analyses into the program and the flight, no preliminary experiments were performed; as a result some unanticipated difficulties arose which caused some samples to be lost.

In addition, the carbohydrate analysis have had low priority; therefore, the data are not refined, in that the results from duplicate aliquots are not as near the same as they should be. Additional sample aliquots will be run, but at this writing have not been. However, it is clear that some of the differences observed are far greater than the analysis variance and are therefore assumed to be real. Unfortunately these real differences appear to be among different carrots, not between treatments.

Tables I, II and III represent the average of two or three aliquots analyses for amylose, sucrose and glucose, respectively. Each is expressed as the per cent dry weight for each carbohydrate. It will be noted that for the initial values, there are duplicates, for two discs were flown from each carrot, but only one additional disc was used for the initial values.

In every sample but one (Table I sample 82-5) there was a utilization of amylose. This ranged to as high as 75% of the original supply in sample 82-3, which was on the centrifuge. It is clear that a general decrease in amylose content occurred, however, there is no indication that the weightless tissue reacted differently than the tissues on the centrifuge.

The sucrose data presented in Table II indicated that sucrose was also lost. Here again there is a large amount of scatter in the data; however, if any trend is indicated it would be that more sucrose is lost from the centrifuged tissue (ave. 67%) than from the weightless tissue (56%).

We had assumed that the disappearance of carbohydrate would represent respiration. Surprisingly, therefore, we found that the concentrates of glucose increased in every case (Table III), usually quite markedly. In looking at the per cent change in concentration, there is a great deal of scatter in these data, and no distinction between the weightless and the centrifuged tissue.

Of greater interest is the absolute final values for glucose. Although there is variability, it might be suggested that all the tissue was trending towards some similar value. Since cells of plant tissue must remain turgid if they are to remain healthy, they must adjust their osmotic potential in the face of environmental water stress. These data suggest that water stress was the overriding environmental factor which was inducing these tissue pieces to increase the proportion of small molecules in their cells, thus permitting them to obtain water, or retain water within their environment.

TABLE I. Amylose as a per cent of the total dry weight of the carrot before and after flight and the change in the per cent amylose

Sample number	Initial	Final	Change
Weightless			
76-1	2.59	1.04	-1.55
76-3	2.59	1.02	-1.57
77-5	3.00	2.19	-0.81
77-7	3.00	1.86	-1.14
78-1	2.99	1.41	-1.58
78-3	2.99	2.23	-0.76
78-5	3.71	2.71	-1.00
78-7	3.71	2.18	-1.53
Centrifuge			
82-1	2.16	0.57	-1.59
82-3	2.16	0.53	-1.63
82-5	2.22	2.29	+0.07
82-7	2.22	1.53	-0.69
84-1	2.39	1.12	-1.27
84-3	2.39	1.21	-1.18

TABLE II. Sucrose as a per cent of the total dry weight of the carrot before and after flight and the change in the per cent sucrose

Sample number	Initial	Final	Change
Weightless			
76-1	5.27	2.79	-2.48
76-3	5.27	1.49	-3.78
77-5	5.08	3.09	-1.99
77-7	5.08	2.16	-2.92
78-1	----	----	----
78-3	5.37	2.06	-3.31
78-5	9.46	4.40	-5.06
78-7	9.46	3.63	-5.83
Centrifuge			
82-1	6.78	0.86	-5.92
82-3	6.78	0.52	-6.26
82-5	8.48	4.88	-3.60
82-7	8.48	2.89	-5.59
84-1	6.42	3.46	-2.96
84-3	6.42	1.82	-4.60

TABLE III. Glucose as a per cent of the total dry weight of the carrot before and after flight and the change in the per cent glucose

Sample number	Initial	Final	Change
Weightless			
76-1	5.04	6.56	+1.52
76-3	5.04	6.16	+1.12
77-5	3.79	8.84	+4.05
77-7	2.79	8.03	+5.24
78-1	5.26	7.94	+2.68
78-3	5.26	9.43	+4.17
78-5	2.99	8.72	+5.73
78-7	2.99	8.75	+5.76
Centrifuge			
82-1	3.21	7.45	+4.24
82-3	3.21	4.83	+1.62
82-5	2.38	9.38	+7.00
82-7	2.38	9.31	+6.93
84-1	4.54	8.30	+3.76
84-3	4.54	8.14	+3.60

As noted above, we had expected that there would be a decrease in total carbohydrate, and that this decrease would represent the rate of respiration. As can be seen in Table IV, some discs decreased in total carbohydrate, while others increased. In addition we expected that most of the dry matter incorporated into the galls would be carbohydrate from the tissue disc. If these added weights are included, there is an indicated decrease in total carbohydrates for even fewer of the tissue discs. Since fructose was not analyzed, we do not know how it responded to this treatment, but if it responded as glucose did, then the reasonableness of the conclusion is increased.

The most reasonable interpretations are that a portion of the non-carbohydrate material, such as protein and lipid was converted to carbohydrate or that a significant proportion of the materials transported to the disc were not carbohydrate or both. And, as mentioned above, the most significant overriding factor of this experiment was the requirement of osmo-regulation by the tissue pieces.

TABLE IV. Change in total amylose, sucrose, and glucose in each carrot disc expressed as mg. Also the net change of the three carbohydrates combined and the gall dry weight

Sample	Δ amylose	Δ sucrose	Δ glucose	Δ total	Dry weight-gall
Weightless					
76-1	-4.54	-7.27	+4.46	-7.35	7.8
76-3	-3.81	-9.16	+2.72	-8.25	4.9
77-5	-2.84	-6.99	+17.78	+7.95	9.5
77-7	-2.92	-7.49	+10.88	+0.48	5.8
78-1	-5.19	-----	+8.82	-----	3.5
78-3	-1.91	-8.31	+10.49	+0.27	14.5
78-5	-3.86	-19.56	+22.16	-1.26	14.0
78-7	-4.98	-18.92	+18.70	-5.20	18.5
Centrifuge					
82-1	-4.40	-21.17	+11.75	-13.82	37.5
82-3	-3.63	-13.93	+3.60	-13.96	13.5
82-5	+0.27	-13.48	+26.21	+13.00	32.5
82-7	-2.52	-20.50	+25.40	+2.38	39.5
84-1	+3.64	-8.40	+10.77	-1.27	2.5
84-3	-2.99	-11.68	+9.15	-5.52	8.9

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RESPONSES OF CROWN GALL TISSUE TO THE SPACE ENVIRONMENT:

TUMOR DEVELOPMENT AND ANATOMY

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ABSTRACT

Statistically significant larger crown gall tumors developed on carrot disks on a centrifuge exposed to the space environment than those in weightlessness. This is the opposite reaction predicted from previous Earth-based gravity compensated experiments. An increase in radius of the meristematic rings of growth centers in these teratoma type galls was observed, however, for tissues generated in weightless conditions.

INTRODUCTION

The crown gall system has been suggested for studies of the long-term effects of weightlessness on biological systems. Tumors induced by inoculating cut surfaces of carrot disks with cells of *Agrobacterium tumefaciens* (E. F. Smith & Towns.) Conn. were significantly larger when gravity compensated on clinostats than those developed at 1 g (9). An increase in radius of the meristematic rings of growth centers in the teratoma type galls (5) was also observed in gravity compensated tissue (3). These effects were ascribed to enhancement of metabolism resulting from more uniform orientation of metabolically active particularates in gravity-compensated cells leading to steeper solute concentration gradients between the particles and the adjacent cytoplasm (1). In this paper we report comparisons of these parameters between tumors developed in the space environment and earth-based controls.

MATERIALS AND METHODS

Carrots were cross sectioned and suspended aseptically, using gnotobiotic techniques (4, 6), into flight canisters (Fig. 1) according to procedures previously reported (9). In these experiments the top surface of carrot disks was the apical facing surface in all cases. Water agar (1.5%) was poured on the Lexan plastic support plates to maintain a high relative humidity and to seal the suspended carrot sections. The upper surfaces of the sections were inoculated with bacterial suspensions (1×10^8 cells/ml) of *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn. grown in Stoner's glutamate medium. Each disk received 0.2 ml of suspension.

Each canister contained three dishes and there were four carrot disks in each dish.

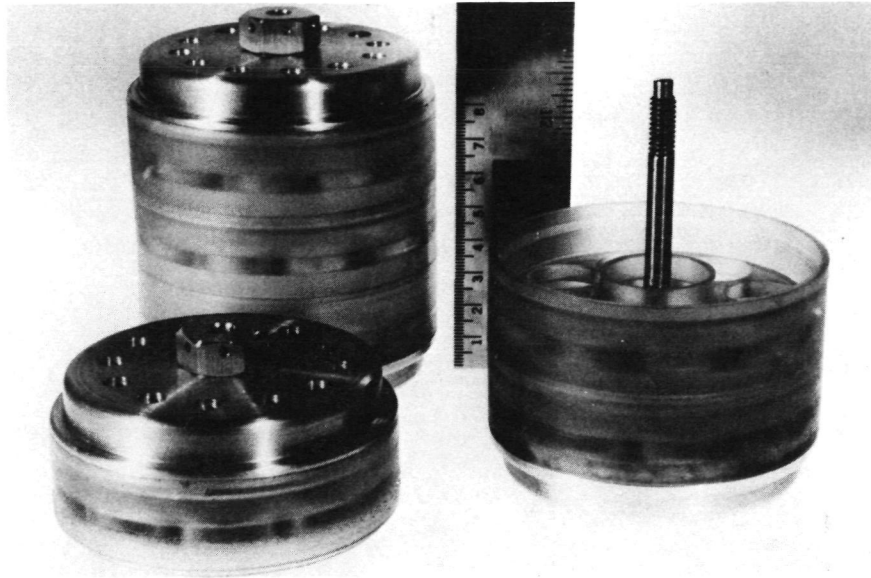


FIG. 1

Flight canister used as a container for carrot disks
exposed to various treatments.

The inoculated disks in the canisters were incubated at 25°C for 72 hrs to allow initiation of tumors. At the end of this period they were placed in chambers maintained at 4°C for transport (8).

Canisters were exposed to the weightless conditions of the space environment (hereafter referred to as the flight treatment) and to the effects of the on-board centrifuge. Parameters of these conditions are described elsewhere (8). Synchronous controls were also run in the USSR. At NASA-Ames, two multiple unit "single axis" clinostats similar to the one illustrated and described by Gordon and Shen-Miller (2) were used to elicit gravitational responses. One clinostat was oriented so that the disks and developing tumors were oriented vertically with respect to gravity (hereafter identified as vertically rotated) and the other was oriented to give horizontal placement and rotation of tissues (hereafter identified as horizontally-rotated or gravity-compensated). The speed of rotation in either case was 1 rpm. Two other treatments were used as nonrotated controls. In one control, tumors were oriented vertically with respect to gravity, and the containers were placed on a table (hereafter identified as vertical-stationary); in the other, the containers were placed on their sides (hereafter identified as horizontal-stationary). Another set of controls were maintained on the ground in Russia. These are hereafter referred to as synchronous controls (8).

The generated tumors and supporting disks were either killed and fixed for histological examination or frozen for subsequent weighing, carbohydrate, or enzyme analyses. Tumors for histological studies were fixed with formalin-alcohol-acetic acid (FAA) or Craf III fixatives. They were dehydrated in an ethanol-xylene series and stained with

safranin O-fast green (7).

Tumors were excised from frozen tissue and weighed. Since frost accumulated on the tissue, dry rather than fresh weights were deemed more reliable indicators of tumor development under the various gravity treatments.

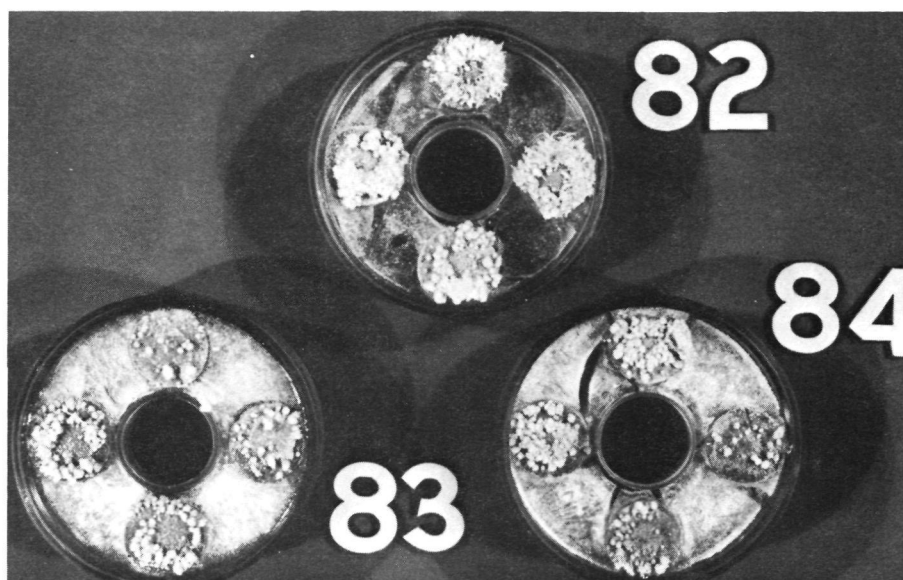
RESULTS AND DISCUSSION

The experimental material was received at the laboratories of Colorado State University. Those generated on the flight were returned in good condition (Fig. 2). Tumors had developed in the synchronous controls but many were discolored (dark pigmentation) and globules of frozen agar (Fig. 3) indicated some type of heat insult had occurred. Tissue returned from NASA-Ames in many instances was dried especially in treatments receiving rotation (Fig. 4).

Tumor weights are given in Fig. 5. Variation was large in synchronous and ground (NASA-Ames) controls. This may have been due to heating and drying of tumors during incubation. No significant statistical differences in weight were noted between any of the controls and flight treatments except in tumors generated on the flight and synchronous centrifuges. Since environmental parameters in the synchronous controls are not known, no conclusions can be drawn from this observation.

Dry weights of tumors generated on the centrifuge in space were significantly larger than those in the weightless environment. This is just the opposite result predicted from previous experiments based on comparisons of tumor weights in gravity compensated and noncompensated treatments (9). Twelve disks in each treatment were used to generate tumors for weighing and, while there was some variation, tissues

A



B

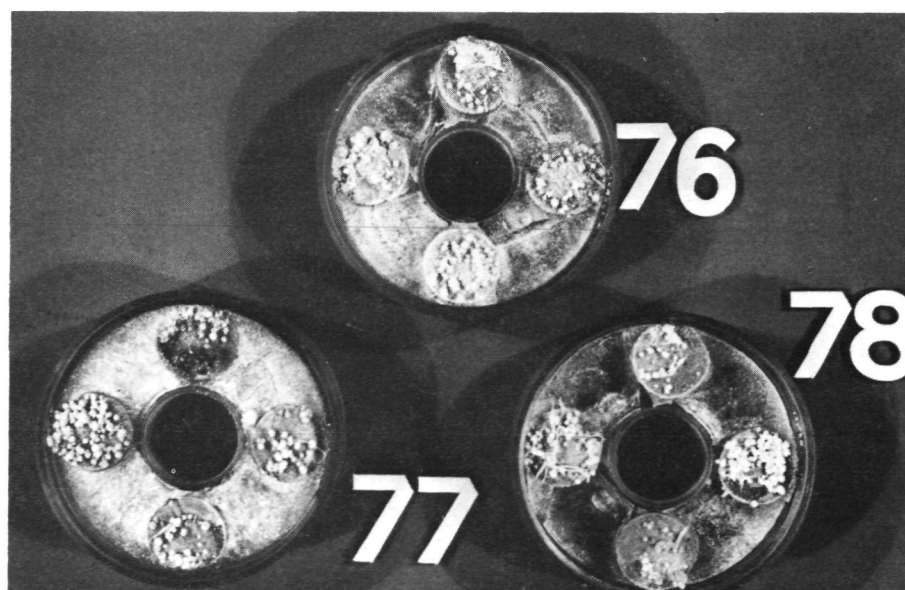
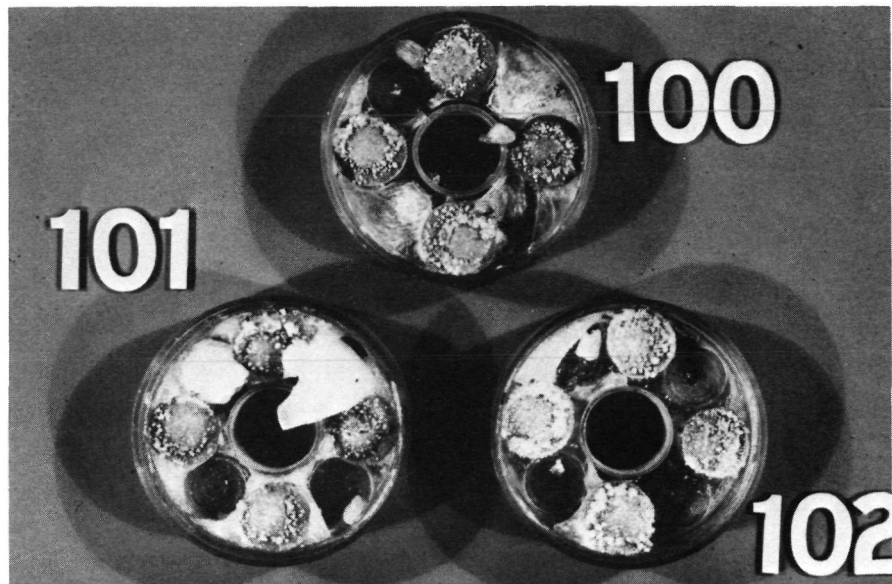


Fig. 2. Tumors generated in space on centrifuge (A) or under weightless conditions (B).

A



B

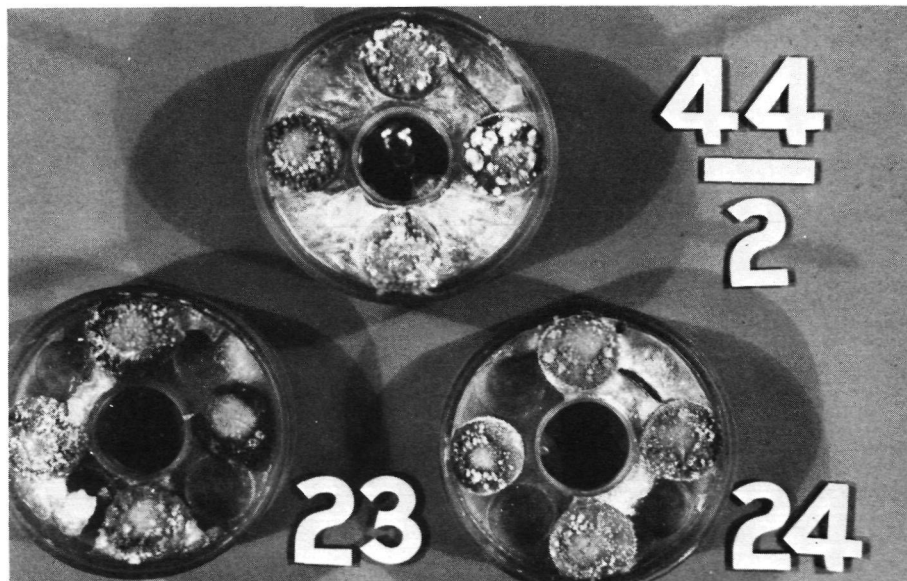


Fig. 3. Tumors generated in synchronous controls on centrifuge (A) or at 1 *g* (B).

A



B

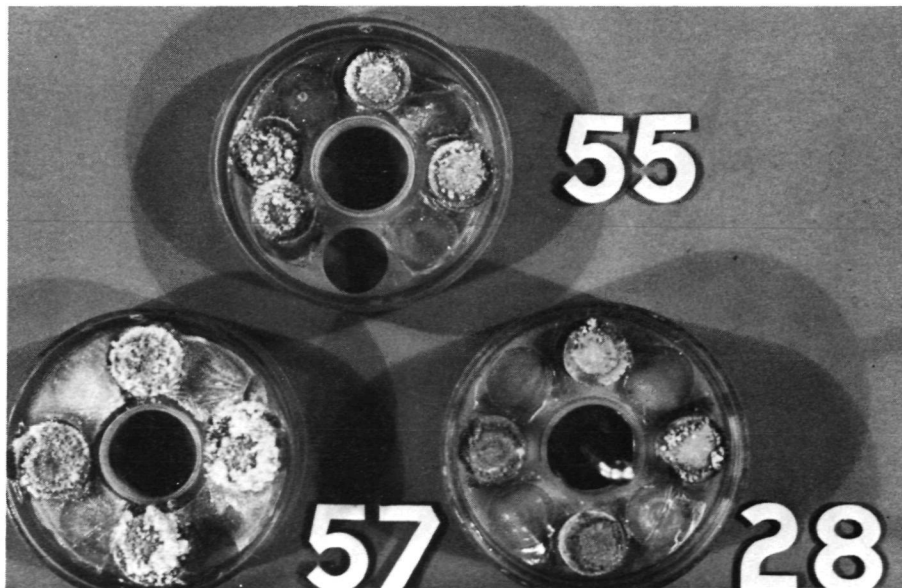


Fig. 4. Tumors generated in controls on clinostat rotated vertically (A) and gravity compensated (rotated horizontally, B).

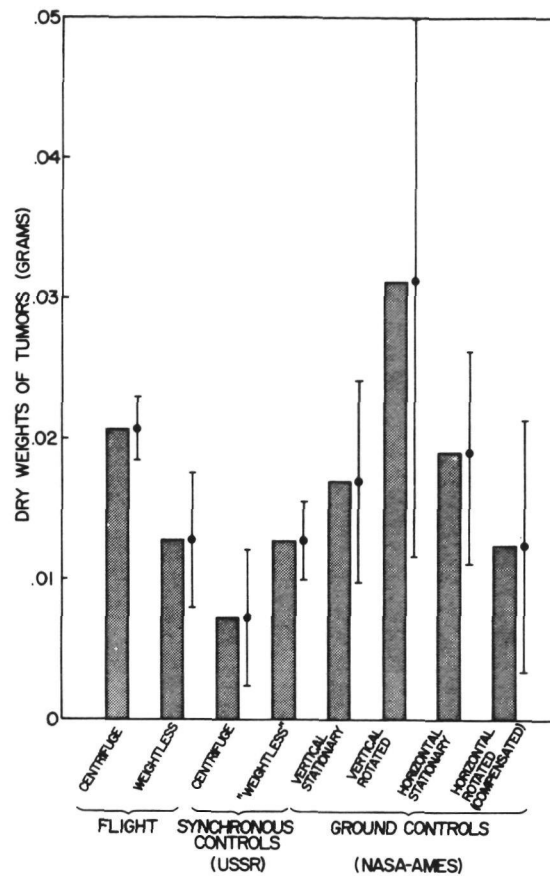


FIG. 5 Mean dry tumor weights of galls removed from carrot disk surfaces exposed to various gravity treatments. The standard deviations shown as vertical brackets were computed from original data.

appeared in excellent condition upon recovery (Fig. 2). It is possible that more disks in the weightless treatment were nonresponsive to generation of galls than those on the centrifuge but plots of individual tumor weights from each disk appear to eliminate this possibility (Fig. 6).

There is currently no body of theory explaining this observation and its validity can only be established in repeat experiments.

There were highly significant differences ($P < 0.01$) in the number of cells in the radius of the meristematic ring of growth centers in tumors (3) between tissues developed at 1 *g* (exposed to earth gravity or centrifuge) and those exposed to the space environment or gravity compensated (Fig. 7). Since there was no significant difference between the weightless flight treatment and the gravity compensated ground controls, in this respect results obtained using a clinostat were similar to those observed in tissues developed in the space environment.

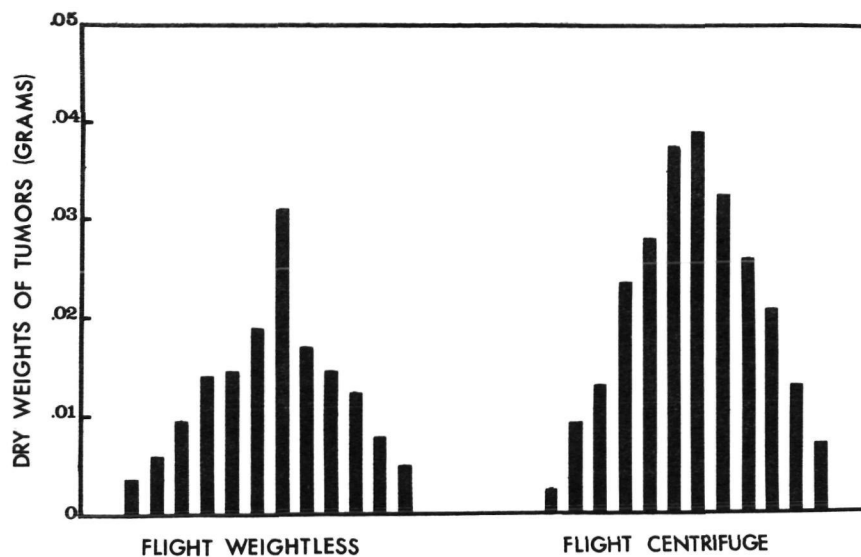


FIG. 6 Distribution of individual tumor dry weights in flight treatments.

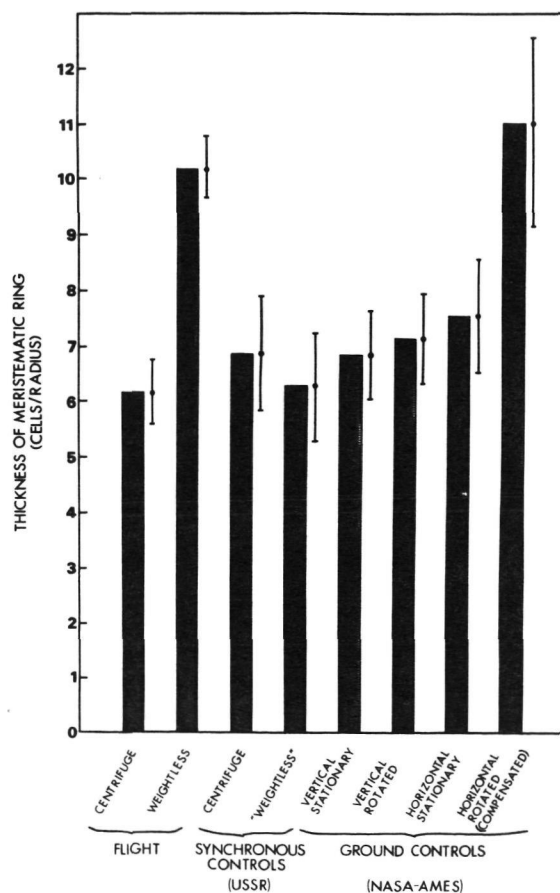


FIG. 7 Numbers of cells of meristematic rings of growth centers in tumors incited by *Agrobacterium tumefaciens* on carrot disks following various gravity treatments. The standard deviations shown as vertical brackets were computed from original data.

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RESPONSES OF CROWN GALL TISSUE TO THE SPACE ENVIRONMENT:

GLUTAMINE SYNTHETASE ACTIVITY

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ABSTRACT

Crown gall tumors exposed to weightlessness, null-gravity experimentation and appropriate controls were analyzed for the specific activity of glutamine synthetase following such treatment. It was found that the specific activities of compensated or weightless material are, in general, lower than the various controls. The data also indicates that null-gravity and true weightlessness, with respect to GS activity, are largely comparable.

INTRODUCTION

Glutamine synthetase (GS) is a rather ubiquitous enzyme found in both the plant and animal kingdoms. In the animal kingdom, this enzyme is generally found with accompanying high specific activity in tissues exhibiting nervous and metabolic functions. In plants, the enzyme is associated with seeds and relatively undifferentiated embryonic activity. It was of interest, therefore, to determine if the specific activity of this enzyme would be affected by null-gravity experimentation and true weightlessness in crown gall tumor tissue. The data obtained would serve as an indicator not only of the comparative differences between normal and pathological material, but also whether null-gravity experimentation is, in reality, comparable to weightlessness.

MATERIALS AND METHODS

Specimens of tumor material were obtained in a frozen condition following various experimental and control maneuvers by NASA and the COSMOS 782 Biosatellite experiments. Thus we had material provided to us which had experienced true weightlessness and a variety of control and simulated stresses. Upon arrival at our laboratories, the experimental material was weighed, frozen, lyophilized and stored at -20° until assayed. Additional experiments (controls and compensated) were performed by us. This material was also weighed, frozen, lyophilized and stored at -20°C until assayed for GS activity. Assays for GS specific activity depended on the gamma-glutamyl-transferase properties of the enzyme and were performed by a sodium arsenate modification of the method of Rudnick et al. (2). The specific activity was calculated as the number of micromoles of gamma-glutamylhydroxymate produced per hour per mg protein. Protein was determined according to the method of Lowry et al. (1).

RESULTS AND DISCUSSION

Following analyses, the data were obtained regarding the specific activity of glutamine synthetase under various experimental conditions (Table 1).

The results obtained from the various NASA and USSR controls cannot be interpreted. It was evident that drying and temperature stresses to varying degrees had occurred. Thus, the only data from ground controls considered reproducible are those obtained from our own experiments.

In this respect, it is seen in Table 1 that there was a 100% difference in GS specific activity between the flight weightless and the flight centrifuge material. Also of importance is the fact that flight centrifuge material revealed greater activity than did flight weightless material. This situation was reversed in our ground controls, that is, our VR material showed less activity than did our compensated HR material. The rationale for these differences would be speculative at this time.

It should be especially noted here, that at no time did the specific activity of flight material or our controls approach the activity of normal carrot tissue and in all cases the activity of rotated or flight material was much lower than stationary controls. The reasons for these differences are not apparent. However, as lack of cellular differentiation or hyperplasia of the cambial zone appears to be characteristic of compensated tumors, GS activity could be expected to be a metabolic participant in neoplastic activity.

The evidence presented above would indicate that specific GS activity is a good measure of weightlessness in this tissue and that null-gravity and true weightlessness under the conditions of these experiments, are largely comparable.

TABLE 1. *Glutamine synthetase activity resulting from various gravity treatments*

	Glutamine synthetase activity		
	<u>um gamma GHA/hr/mg protein</u>		
	Run I	Run II	Ave
Synchronous			
Centrifuge	0.56	0.54	0.55
"Weightless"	0.87	0.64	0.76
NASA-Ames Controls			
Horizontal Stationary	0.58	0.06	0.59
Horizontal Rotated	----	1.36	1.36
Vertical Stationary	1.16	0.79	0.97
Vertical Rotated	0.76	0.82	0.79
Flight			
Centrifuge	0.20	0.23	0.21
Weightless	0.10	0.11	0.11
CSU Controls			
Normal Carrot	0.73	0.91	0.82
Horizontal Stationary	0.54	0.64	0.59
Vertical Stationary	0.48	0.56	0.52
Horizontal Rotated	0.35	0.37	0.36
Vertical Rotated	0.25	0.35	0.30

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RESPONSES TO CROWN GALL TISSUE TO THE SPACE ENVIRONMENT:

ISOZYME PATTERNS

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ABSTRACT

Peroxidase zymograms of gravity-compensated crown gall tissue were compared with those of tissue actually flown at zero gravity. The patterns obtained, while not identical, were distinct from control tumors grown at one g. on the spacecraft or kept stationary in the laboratory. The results obtained support the previous study which showed that the gravitational field influences isozyme activity in carrot crown gall.

INTRODUCTION

Clinostats have been used to simulate conditions of zero gravity. Carrot crown gall tumors were reportedly larger with respect to both fresh and dry weights when grown under gravity-compensated (horizontally rotated) conditions on a clinostat (3). A previous report also showed that such tumors display a peroxidase zymogram which differs from that of vertically rotated or stationary controls (2). Esterase zymograms, however, were identical in vertically and horizontally rotated tumor tissues.

To compare the efficiency of clinostats in duplicating true zero gravity in the space environment, carrot discs inoculated with the tumorigenic agent, Agrobacterium tumefaciens, were flown on the Soviet Cosmos in November, 1975. This report describes a comparison of peroxidase zymograms between tumor tissue grown in space and that grown on land-based clinostats.

MATERIALS AND METHODS

For a detailed discussion of the protocol for preparation of carrot discs, inoculation and environmental conditions during growth see the previous papers (2) and (1).

The following treatments were studied: flight weightless, flight centrifuge (to simulate 1 g), clinostat—horizontally rotated (gravity-compensated) and horizontal stationary (1 g). The synchronous ground controls (clinostat and stationary conditions) kept in Moscow and at NASA Ames were too desiccated for use: thus a separate set of tissues kept at Colorado State University was used.

Peroxidase isozymes were visualized with benzidine as previously described (2).

Sufficient material was available for only one extraction and three separate electrophoretic runs. The results were not identical on each run; therefore an "average summary" is presented.

RESULTS

Only five major bands of peroxidase isozymes were detected in this study. The activity of each band was dependent upon the conditions under which the tumors were grown. Both the flight weightless tissue and the clinostat gravity-compensated tissue had a band at the third position which stained intensely. The two treatments were not identical, however, since the gravity-compensated tissue also contained an isozyme with intense activity at position one.

The flight centrifuge tissue and the ground-based horizontal stationary tissue had in common an intensely-staining band at position 2. The horizontal stationary tissue had in addition a strong band at position 4.

An interpretive drawing of the combined results is shown in Fig. 1. and an actual photograph of one of the electrophoretic runs is shown in Fig. 2.

DISCUSSION

Eight major bands of peroxidase activity were reported in a previous paper (2) whereas only five were found in the present study. In addition, the zymograms of the gravity-compensated and stationary controls are different from the same treatments studied in a previous paper (2). Experimental modifications for the present report may account for the

FIG. 1

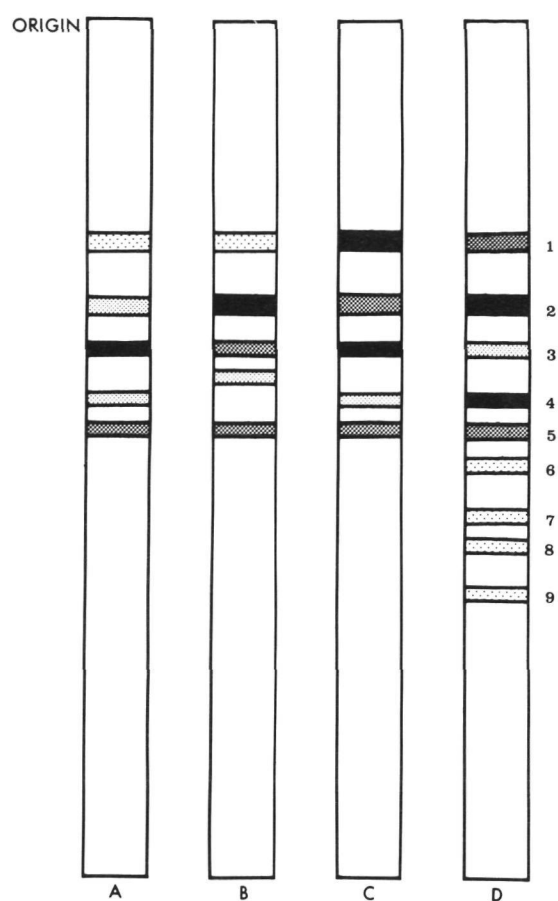


FIG. 2

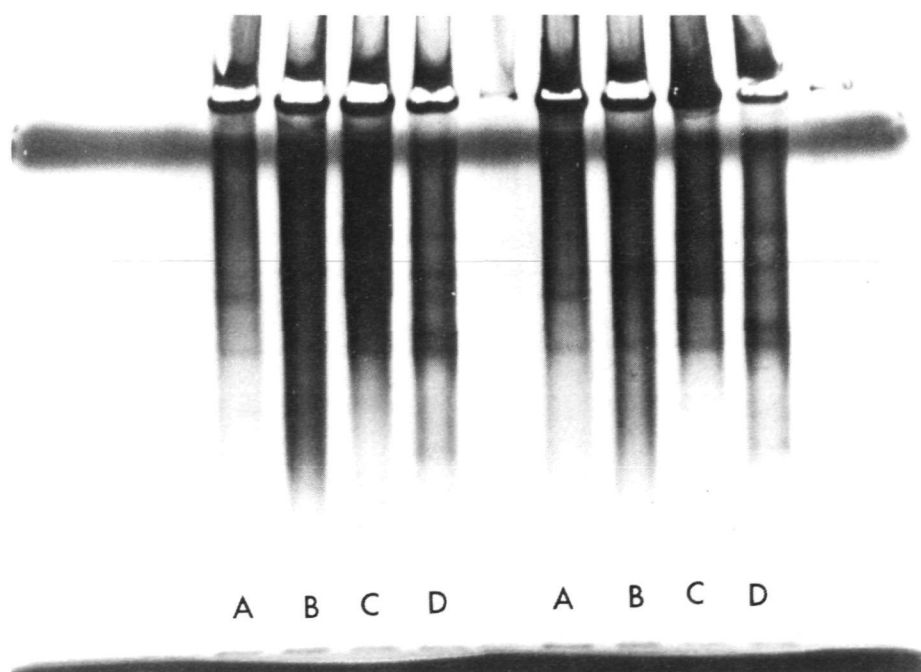


FIG. 1 and 2. Peroxidase isozymes from carrot crown gall.
 FIG. 1. Interpretive diagram of combined experiments.
 FIG. 2. Sample photograph of one electrophoretic run. A: Flight weightless, B: Flight centrifuge, C: Horizontal rotated, D: Horizontal stationary.

changes. The composition of the acrylamide gel was slightly modified for these experiments in order to enhance resolution of the bands. Secondly, all material used in the previous report was extracted from fresh tissue whereas all samples were freeze-dried prior to extraction in the present report.

The results show both a clear similarity and a clear difference between the true weightless and simulated weightless treatments. The same results were apparent in the flight-one gravity and ground based stationary control. When the appropriate pairs of treatments were compared, however, they did fall into two logical groupings: gravity compensated and one g.

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THE MORPHOGENETIC RESPONSES OF CULTURED TOTIPOTENT CELLS
OF CARROT (Daucus carota L.) AT ZERO GRAVITY

A FINAL REPORT OF WORK ON COSMOS 782 (NASA CONTRACT NAS 2-8986)

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SUMMARY

A "carrot cell culture experiment" was aboard the unmanned Soviet Vostok biosatellite (Cosmos 782) launched on November 25, 1975 in the Soviet Union. The principal mission of this experiment was to test whether cells of a higher plant, such as the carrot, could develop normally under conditions of weightlessness in space and emulate their known ability on earth to multiply and develop, forming organs, embryoids and normal plantlets. The experimental system made the test more feasible because free cultured somatic carrot cells can develop like zygotic embryos and can initiate the complex growing regions that produce organs (shoots and roots) independently of the normal environment of the fertilized egg.

The Soviet satellite carried cells in plastic petri dishes encased in special canisters and for 19.5 days in space exposed them to both weightlessness (zero-g) and, by means of a centrifuge that was on board the spacecraft, exposed them also to a gravitational force equivalent to that on earth (1-g).

For the purposes of this experiment carrot cells were pregrown in liquid suspension cultures and brought to the appropriate level of activity in growth and morphogenetic propensity. The cells in question originated from somatic cells of seedlings of the wild carrot (Daucus carota or Queen Anne's Lace) and they had been pregrown through many transfers and cell generations in flasks being slowly revolved about a horizontal shaft so that they had not been exposed to asymmetric gravitational stimulus during their random proliferative free growth. The cells so

prepared, totipotent in the sense that like zygotes they can give rise to complete plantlets, had no visible characteristics of the differentiated mature somatic cells of their origin. The petri dishes contained actively growing cultured cells all of which were, by filtration through graded sieves, less than 74 μm in size; these cells were distributed in thin layers in an agar culture medium which allows them to grow heterotrophically in darkness.

Much prior work was necessary, and is described, on various clones of carrot cells to demonstrate their ability to develop organized embryonic forms in 19.5 days in darkness and in the surface of the thin layer of agar medium contained in sealed plastic dishes. By suitable tests it was demonstrated that cultures so prepared could withstand the physical hazards of lift-off, flight and recovery. Devices used to defer all biological activity in times of transit to and from launch and recovery sites are described as they were both devised and tested. To ensure additional controls for the experiment, dishes comparable to those in flight were maintained at NASA Ames Research Center, California, at State University of New York at Stony Brook, and also in the Soviet Union. The plan was also to expose ground control dishes in the USSR to conditions that simulated the circumstances of lift off and conditions within the spacecraft except zero-g. The U.S.A. controls also included dishes placed on a klinostat rotating at 1 rpm; both so-called "horizontal" and "vertical" klinostat controls were carried out. Canisters were also kept at $4 \text{ degrees} \pm 2^\circ\text{C}$ for the entire period following preparation of the cells at Stony Brook until just before launch in the USSR and until "synchronous controls" were performed. It had been determined by prior experimentation that such chilling precludes cell division and development, and it was anticipated that once on the spacecraft, and at the temperature

of flight (20-24°C) metabolic activity and growth would resume and morphogenetic potential could be expressed inasmuch as conditions in flight permitted.

In the final outcome, viable biological material was recovered from all treatments except those that were to simulate lift-off, flight and recovery on the ground in the U.S.S.R. However, as the complete record (based on photographs of the dishes, the number of developed structures they contain, photographs of "embryos" at different stages of their development) shows, the useful result is that even the dishes exposed in flight at zero-g produced viable embryos in large numbers, that they did not differ significantly from those exposed in flight to 1 g and, in consequence, the various other controls were not needed to test the reality of any observed quantitative differences between the responses observed at zero-g and 1 g in flight.

The particular clone of carrot cells used contained units which, under the conditions and duration of the experiment, varied greatly in the level of development they achieved. These were arbitrarily classified by size and complexity into many categories and their frequency in the populations on the dishes was established by actual counts. Representative samples of the different stages of developed embryos were obtained for photography and microscopic examination. However, the large numbers of initial units on the dishes and the number of replicate dishes enables it to be established that the 0 g and 1 g treatments did not result even in significantly different proportions of embryos at the different stages of development into which they were classified.

The level of development achieved from free single cells at 74 μ or less in 20 days in darkness produced embryos at various levels of heart and torpedo and early cotyledonary development. Where embryos were larger, their increased size or complexity was in the length of primary root (which could be substantial) and in the development of hypocotyls. Shoot development tended, however, to be arrested. Nevertheless, the leaf primordia and shoot apices had become sufficiently established during the experiment, at both 0 g and 1 g, so that when embryos from flight were exposed to 1 g and to light their shoots developed rapidly and normally into plantlets. Such plantlets have been carried on in the greenhouse and will be grown to maturity.

The conclusion, therefore, is that totipotent somatic cells can undergo morphogenesis to produce viable and fully competent embryos at 0 g, apparently as effectively as at 1 g. There are, however, two reservations. First the imposed conditions (i.e. total darkness) and duration of the experiment do not preclude the possibility that the later rapid development of shoots with the concomitant tissue differentiation might be more sensitive to 0 g than the initial induction of embryonic form. Secondly it is still an open though perhaps unlikely question whether the cells as used in the experiment had through their pre-treatment retained some consequences of asymmetric 1 g stimulus so that, when they did develop at 0 g in space they could respond as to a "1 g presentation time" i.e. to an existing "memory" of prior 1 g conditions. These are, however, considerations for further experiments that are discussed.

Introduction

The classical problems of embryonic induction, i.e. the causal factors that control the means by which zygotes give rise to embryos with a full complement of differentiated tissues and developed organs, are as present today as when they were first enunciated. Modern understanding of the molecular basis of inheritance, without comparable knowledge of the mechanism of localized controls over gene expression, has not yet disposed of the problems of morphogenesis. Higher plants, which produce their embryos from zygotes in the embryo sac and ovule, under the specialized nutritional and physical conditions of these environments, still present the problems of morphogenesis in dramatic form. Nevertheless, the demonstrable ability of freely cultured somatic cells of angiosperms to undergo a simulated zygotic embryogenesis now permits these problems to be investigated apart from the immediate complications of sexuality and fertilization. In so doing, the opportunity also arises to design a test system in which somatic cells may develop into embryos under conditions that can be duplicated in outer space at near zero gravity. The aim of this system was to test whether embryogenesis is in any way dependent upon, or conditioned by, the gravitational stimulus to which plants have been inevitably and universally exposed during their evolution (cf. Wunder et al, 1968a; Gordon and Cohen, 1971).

The purely directional gravitational stimulus (geotropism) which operates on the growth of pre-formed plant organs that have acquired the ability to perceive and respond to gravity during their development at 1 g are familiar (Anker, 1962; Audus, 1969; Ball, 1969; Larsen, 1962a and b, 1973; Westing,

1964; Shen-Miller and Hinchman, 1974; Wilkins, 1975). But the morphogenetic and physiological propensities of plant cells that have been removed from pre-formed organs, cultured in isolation, and allowed to develop in a gravity-free environment raises problems to which there are no precedents. Here it is important to distinguish between the responses to zero gravity of an initially free cell system as it develops into a multicellular organized structure and those more familiar situations in which pre-formed, gravity-sensitized organs and growing regions have continued their activities for very short periods under zero gravity conditions (cf. e.g. Conrad, 1968; Gray and Edwards, 1971; Lyon, 1968 and 1971; Johnson and Tibbitts, 1971) or those cases in which the directional effects of gravity are neutralized or compensated on earth by rotation around a horizontal axis, as on clinostats (cf. Gordon, 1963; Dedolph et al, 1967; Wunder, et al, 1968a and b; Conrad and Yokoyama, 1971; Gordon and Shen Miller, 1971; Brown et al., 1974). The objective of the investigation to be described was to put these questions to experimental test.

An opportunity to ascertain whether free cells of carrot, capable of organized development, could undergo normal embryogenesis at zero gravity materialized when the Soviet Union invited the National Aeronautics and Space Administration to propose some biology experiments that could be carried on a Soviet Vostok biology satellite scheduled for launch in the last quarter of 1975 (cf. Aviation Week and Space Technology, Dec. 16, 1974 p. 22 and September 22, 1975 p. 20). This report provides a detailed consideration of the experimental system as used, with a description of the observations as made and an assessment, so far as is currently

feasible, of their significance. In the performance of this experiment, the authors enjoyed the help and cooperation of the Institute of Biomedical Problems, Ministry of Health of the U.S.S.R. (Dr. Oleg G. Gazenko, Director) and the Timiriazev Institute of Plant Physiology (Dr. A.L. Kursanov, Director) and especially Dr. Raisa G. Butenko, our scientific counterpart in the U.S.S.R..

MATERIALS AND METHODS: THE TEST SYSTEM

The test system adopted emerged from successive works done since 1947 on the induction of rapid growth in small aseptic isolated explants of the secondary phloem of roots of cultivated carrot; on the combinations of growth factors, nutrients, and conditions, that provide for the most rapid proliferative growth in liquid at 21°C in diffuse continuous light; on the release of viable cells from such cultured explants and their continued subculture; on the development from the repetitively subcultured free cells of organized growing regions, namely roots, shoots, and all the organs of a complete embryonic carrot plant; and, finally, on the demonstration of somatic embryogenesis which simulates zygotic embryogenesis especially closely and profusely when it arises from cells that had originated from seedlings, or even from embryos from immature seeds (cf. Steward, Mapes, Kent and Holsten, 1964; Steward, Kent and Mapes, 1966; Steward, 1968; Steward, Mapes and Ammirato, 1969; Steward, Ammirato and Mapes, 1970; Steward and Krikorian, 1971 or Krikorian, 1975 and references there cited for a comprehensive bibliography).

From all this prior work it was known at the outset that the responses of carrot cells to different combinations of growth-promoting stimuli vary with their origin at different locations in the plant body (cf. Fig. 6 of Steward, 1970)

and, to some extent, with the vegetative clone of their origin. Also, to bring some recalcitrant cells from different plants to vigorous growth and embryonic development, sequences of inductive stimuli, in the form of different combinations of synergistically active substances, may need to be employed (Steward, Kent and Mapes, 1967). Moreover, it was known, that over and above the growth regulatory stimuli derived from exogenous hormone-like substances, the cultured cells (especially in the case of carrot) also react morphogenetically to changes in the external environment (cf. Steward, Ammirato and Mapes, 1970; cf. also Fig. 18 of Steward, 1970 and Fig. 6-3 of Steward and Krikorian, 1971). It is into this complex of interactions that the zero gravity environment introduces a new parameter with unexplored possibilities.

Hitherto, the conditions deemed best for demonstrating the inherent totipotency of living but mature cells of carrot resulted from: -

- a) the use of cells in repeatedly subcultured populations grown in liquid and so filtered that the inocula consisted of single cells, or small clusters of only a few small cells.
- b) the growth and development of the free cells or small units in diffuse light and in liquid media in a total osmotic concentration which is compatible with the development of small embryonic forms (Ammirato and Steward, 1971).
- c) relatively unrestricted and protracted periods for the embryonic development which allow for new cells to be "rubbed off" from developed small plantlets to then re-embark upon embryogenesis

in the medium which had both supported the first profuse crop of plantlets and, in turn, had been "conditioned" by their development.

However, for the purpose of a decisive test whether embryogenesis of an angiosperm can occur in space at near zero g as distinct from the conditions under which gravity is neutralized, as in cultures slowly rotated about a horizontal axis, a different set of limitations were imposed by the conditions in the spacecraft. These limitations which had perforce to be recognized at the outset were:-

- a) the morphogenetic development should take place preferentially on, or in, a shallow layer of semi-solid medium (solid enough to retain its form and dilute enough to permit development to proceed).
- b) the test system to be used should consist entirely of free cells, or small units, and therefore be entirely free from growing regions such as buds or primordia of apices of roots or shoots that had already organized under 1 g conditions prior to the experiment.
- c) at the time of their use, the readied populations (pregrown in liquid) should yield on filtration, the most uniform and the smallest viable cells possible which, when distributed in the test semi-solid medium, would grow and develop with a high "plating efficiency."
- d) at the appropriate temperature the populations of cells to be tested should be capable of the maximum possible amount of organized development within the relatively short period of the actual test (which eventually turned out to be 19.5 days).

MATERIALS AND METHODS: EXPERIMENTAL DESIGN

i) Origin of the cultured cells. Embryos of wild carrot (Daucus carota L. i.e. "Queens Anne's Lace") were removed aseptically and intact from mature seeds collected from plants growing on the Stony Brook campus in August, 1974 and grown in 1 oz. bottles ("French squares") for a period of two weeks in darkness at 22°C on a half-strength modified White's medium (hereafter abbreviated as B_W) (cf. Steward et al, 1975 for details) containing .5% sucrose and .5% agar. The plantlets so obtained were then cut into cm long segments and inoculated directly into culture flasks (cf. Fig. 22 of Steward et al, 1975). About 8 seedlings were utilized per flask--containing 225 ml of B_W + coconut milk¹ (CM) 10% + naphthaleneacetic acid (NAA) 2 mg/liter + casein hydrolyzate (CH).02%. These explants gave rise to vigorous suspensions of cells within a period of 3-4 weeks, and were transferred approximately every 21 days. These transfers were routinely made by passing the contents of individual flasks first through a single layer of cheesecloth and then through a double layer of cheesecloth, allowing the strained material to settle for a period of 30-60 minutes and then decanting the supernatant until a thick suspension (ca. 50 ml) remained. Five ml aliquots of this suspension were then used to inoculate culture flasks containing fresh medium (cf. Fig. 1, especially details on left). After 21 days, suspension cultures so grown were filtered through stainless steel sieves of known dimensions to obtain the cells for experimentation. These cells grown in a medium lacking NAA and coconut milk (i.e. the basal nutrient medium described

¹ Coconut milk, or Coconut water, i.e. the liquid endosperm from the central cavity of mature nuts.

by Murashige and Skoog (1962), hereafter designated as B_{MS} , and inositol 20 mg/l and sucrose 3%) yielded organized plantlets. Such plantlets could be used again as sources of explants to reinoculate fresh flasks so as to maintain stocks with a high level of morphogenetically competent cells. Flasks were rotated (1 rpm) in darkness on apparatus similar to that used for the culture of carrot explants (cf. Steward, Caplin and Millar, 1952) but these were housed in Model M-2 Environmental Growth Chambers, Chagrin Falls, Ohio for better control (22°C and 50% R.H.).

ii) Preparation of cells for distribution in agar. On Wednesday, November 19, 1975 twelve culture flasks were processed by individually passing their contents through a single, followed by a double, layer of cheesecloth. The filtrate was then promptly passed through a stainless steel sieve (#100 mesh = 140 μ m pore size); the filtrate so obtained was then passed through a second stainless steel sieve (#200 mesh = 74 μ m pore size). Fig. 1 shows schematically the essentials, but not all the exact details, of the procedure. Figure 2a, b and c show the appearance of the cells after each of the filtration procedures and Figure 3 shows the subsequent growth of colonies after similar cells from different but equivalent filtrations were grown in replicate dishes. For the purpose of this experiment, it was essential to use cells which had no visible organized development. Cells were washed and collected in beakers and transferred to 35 ml. conical centrifuge tubes with metal closures and then centrifuged at 300 rpm for 10 minutes in a PR-2 centrifuge at ambient temperature. The supernatants were decanted from all 12 tubes and the cells were resuspended

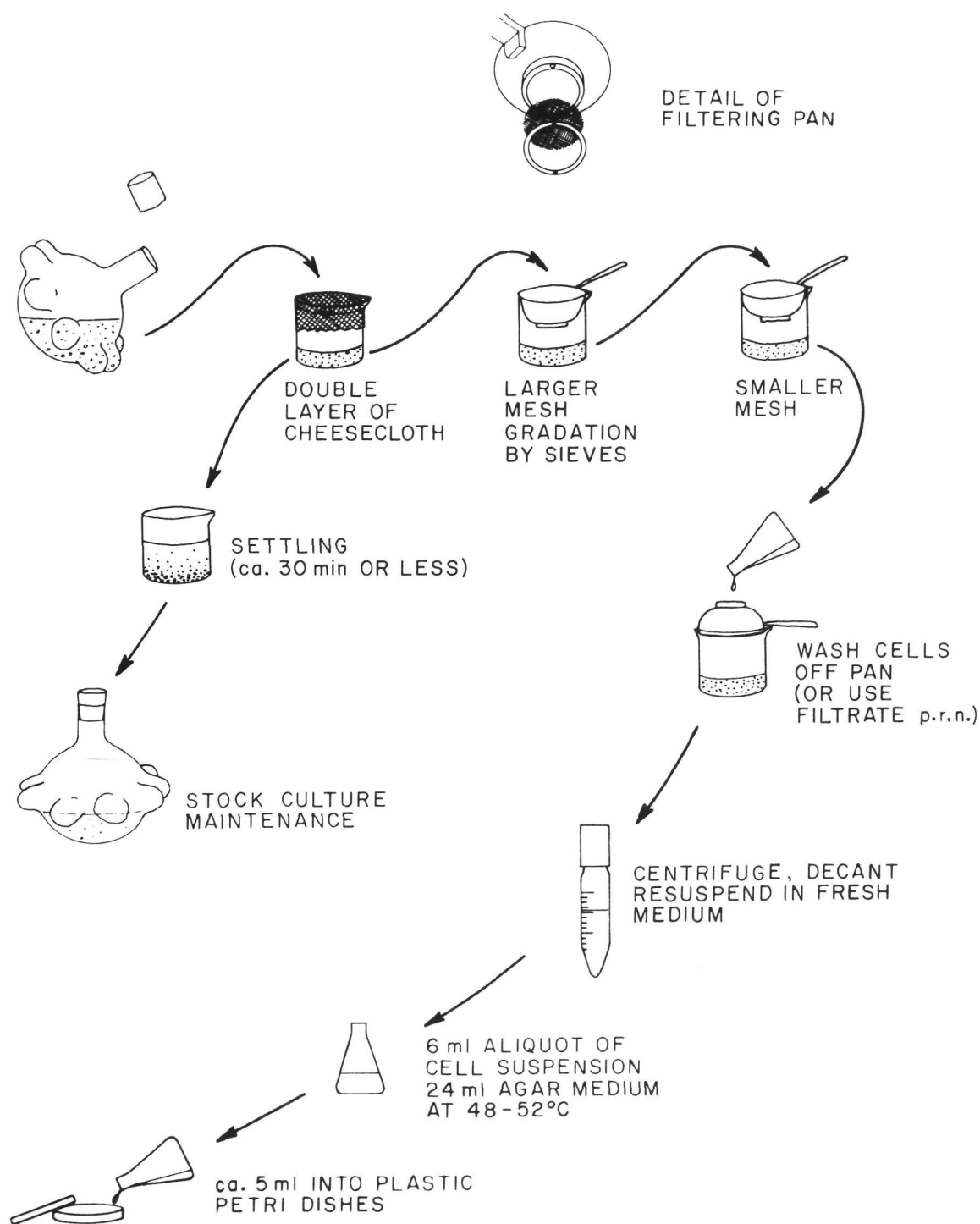


Fig. 1. Diagramatic representation of procedures followed in filtration of cultured cells grown in suspension as to unit size for plating in semi-solid media. The left-hand portion of the scheme also shows a serial transfer being made to maintain stocks.

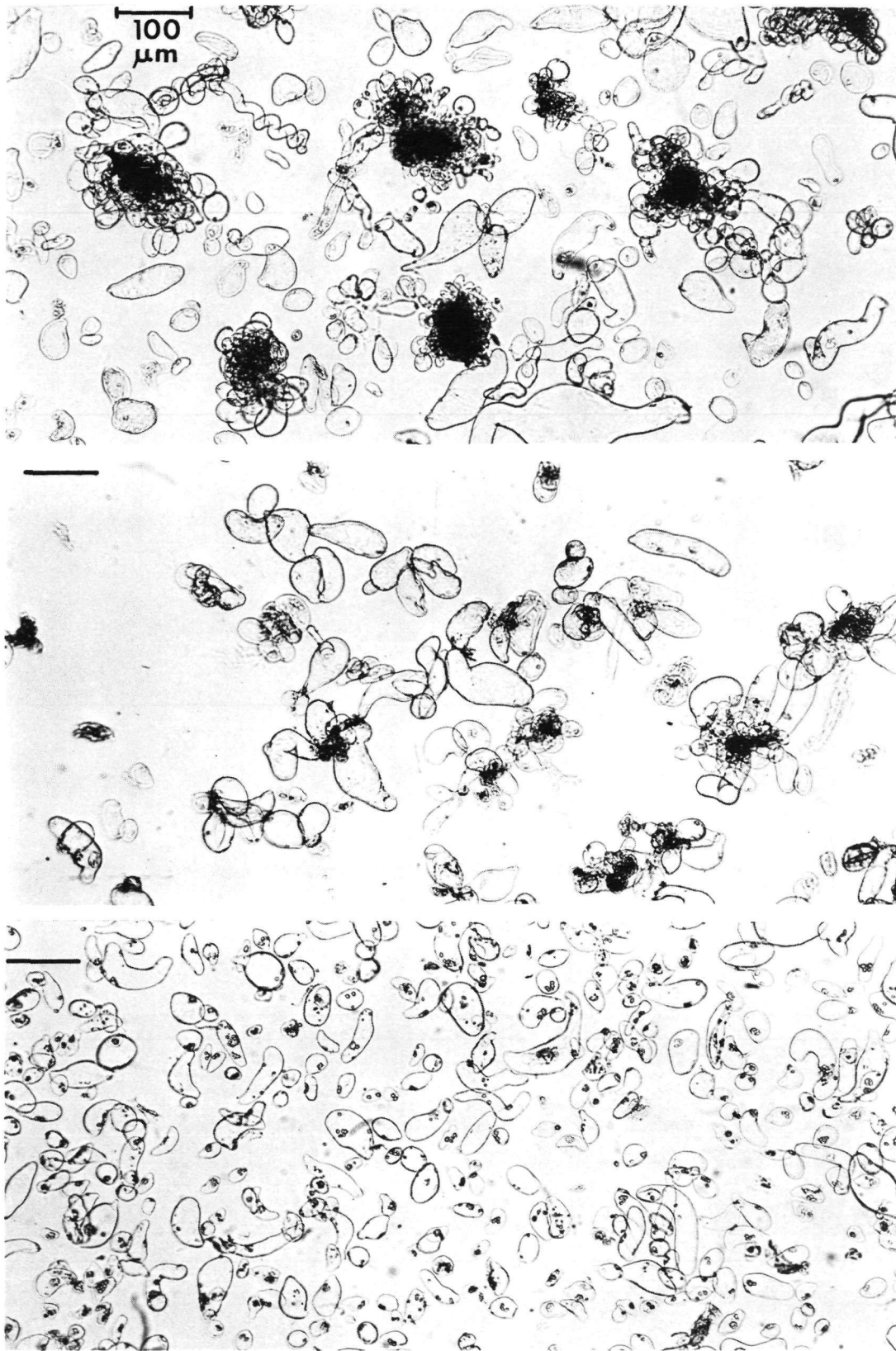


Fig. 2. General appearance of cells from suspension cultures of wild carrot after successive stages of gradation through filters with decreasing pore size. a) after passage through a double layer of cheesecloth; b) after filtration through a #100 mesh sieve (140 μ m pore size); c) after passage through a #200 mesh sieve (74 μ m pore size). Note that most of the cells in c are free.

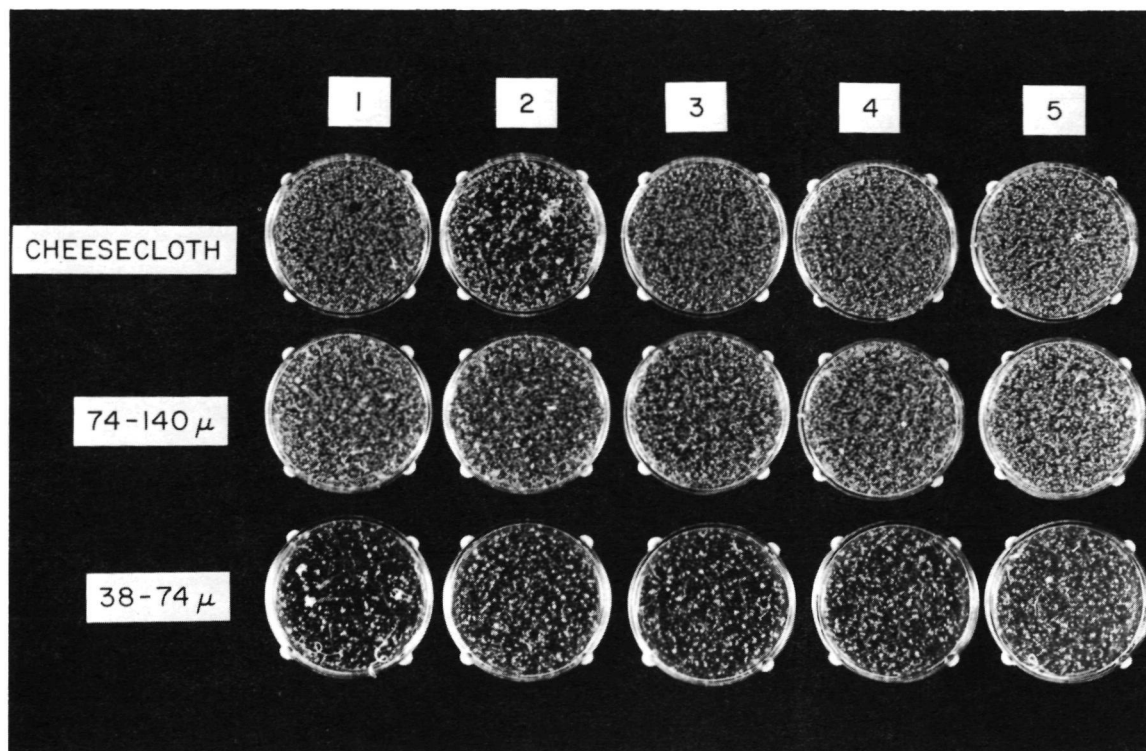


Fig. 3. Organized growth in a medium which supports morphogenesis when the units of the inoculum were graded as to size by the use of 3 "sieves." Material was collected by successive use of a double layer of cheesecloth, followed by sieves numbered #100 and #200 which gave fractions with particles 74-140 μ m to less than 74 μ m. Note that vigorous growth occurred in each case, including that using inocula of essentially isolated cells on very small units.

in approximately 10 ml. of B_{MS} + inositol 20 mg/l + 3% sucrose. The cells were finally combined in a pre-calibrated 500 ml. Erlenmeyer flask. A 5 ml. aliquot was removed by pipetting for determining cell density and for photography. The final volume of cell suspension was adjusted with basal medium to a volume which yielded per ml 41,667 units of which 36,583 were single cells. The cell suspension so adjusted to volume was mixed thoroughly and evenly distributed among eight 35 ml. conical centrifuge tubes.

iii) Plating of the Cells. The plating medium consisted of B_{MS} + inositol 20 mg/l and 3% sucrose. Platings were done in units of 6 plastic petri dishes (50 mm in diameter, Falcon No. 1006). To this end, 50 ml. Erlenmeyer flasks were prepared containing 24 ml medium. Six mls of cultured cell suspension were pipetted, using wide-mouthed pipettes into the 24 ml. of medium after it had been allowed to cool to approximately 48 C. (Although this temperature was subjectively determined, it is very accurate in the hands of an experienced investigator.) The cells were distributed thoroughly throughout the medium by gentle swirling. Approximately five mls of this preparation were then introduced into each of 6 petri dishes (cf. Fig. 1). The dishes were covered by "snapping on" the lids and were then set aside and the agar allowed to solidify. A total of 180 petri dishes were prepared and randomly mixed and stacked into groups of 9; 126 were labelled with a carbide or diamond marker pencil. The remaining 54 provided "Unofficial" material for subsequent observations. Dishes were numbered so that number 1 was at the top of each stack, and number 9

was at the bottom. The experimental containers, here designated as "canisters" consisted of a central acrylic tube with anodized aluminum alloy end-caps. Twelve openings, .250 inches (6 mm) in diameter enable air to enter. Canisters were arbitrarily pre-embossed to facilitate record keeping with the designations, KF-1 through KF-14 (see Fig. 4).

iv) Disposition of plastic petri dishes amongst the so-called "Flight Canisters".

The experimental canisters were loaded by placing stacks of 9 petri dishes each on the so-called "microsil standoffs" (about .063 inch thick) and were then introduced into the acrylic tube which is about 2.5 inches x 4 inches in length. Pyrell foam (# 4 density) was placed on the top of each stack and two H.E.P.A. (glass fiber, about 25 mil thick) filters were placed inside each end-cap (cf. Fig. 4). The end-caps were connected and manually tightened. The canisters were refrigerated at 4°C until transport to Ames Research Center (ARC), Moffett Field, California.

v) Logistics of Transport

Inherent in the design of the experiment were periods, necessarily somewhat long, during which the cells selected for the actual flight had to be held under conditions such that they remained viable but did not embark prematurely upon their morphogenetic development. In particular, these periods covered transit from Stony Brook to Moffett Field to Moscow to the launch site and ultimately back to Moscow and the U.S.A. (both Stony Brook and ARC).

The principle used here was to depress the growth activity of the cells prepared in the dishes as for flight

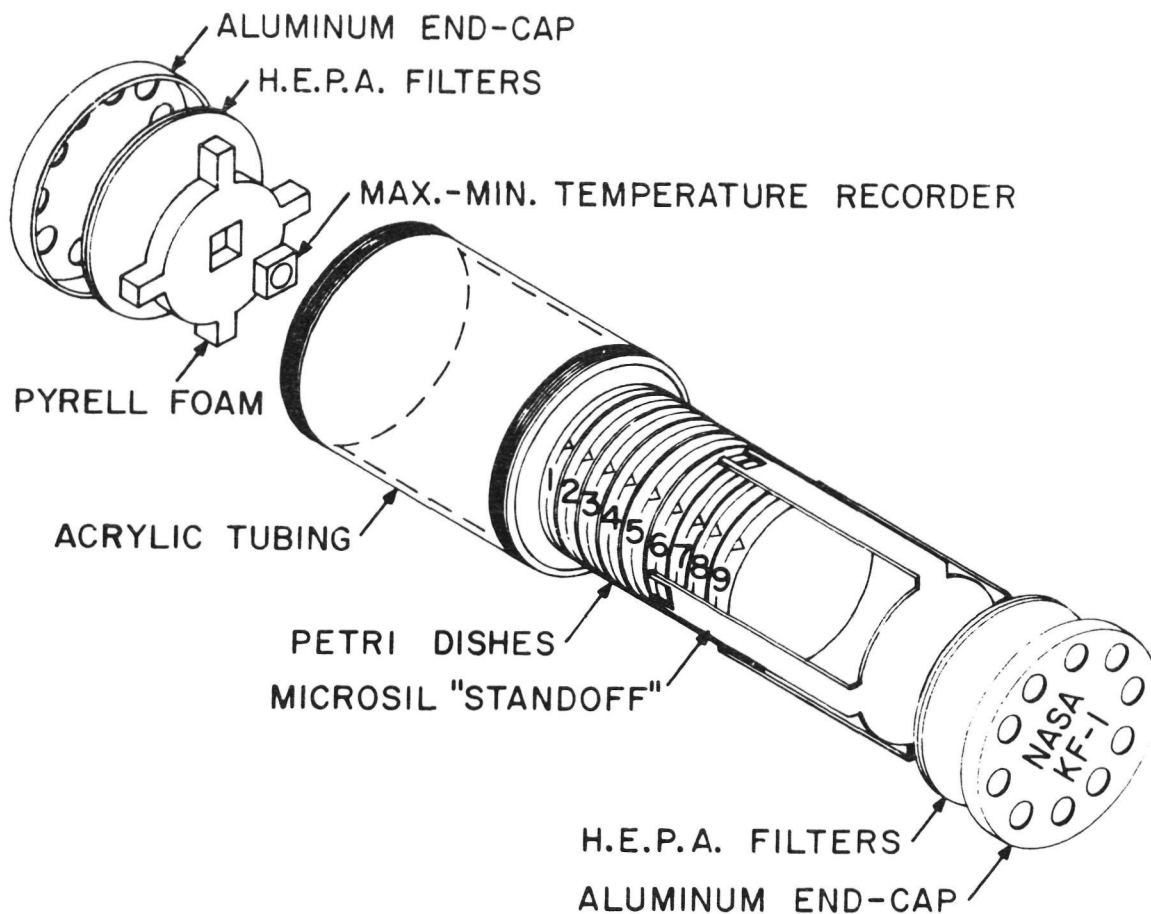


Fig. 4. Diagrammatic representation of a flight canister in disassembled perspective showing petri dishes etc. The numbers on the petri dishes represent position in the canister, 1 is at the top of the canister while 9 is at the bottom.

by lowered temperature. (This is preferable to other means such as modifying the ambient medium). While prior evidence showed that temperatures of about 4°C reversibly restrained the activity of the cells, extensive tests were done to demonstrate that this restraint could last for periods long enough to permit the needed transit of the cultures. Also, these pre-flight trials had to test whether the cells as shipped from Stony Brook to ARC could survive the hazards of simulated lift-off, flight and re-entry, as well as their subsequent return transport to Stony Brook. To make all these tests possible, dishes with cells were shipped in wide-mouth Thermos ® bottles to and from Moffett Field by commercial airlines, usually as "priority parcels." These parcels were not subjected to x-ray. On the basis of this experience, and for later use in the actual experiment, insulated boxes, the so-called "biotransporters" were designed at ARC and equipped with both coolant and battery powered heaters to permit temperature regulation over long periods at 4°C ± 2°C. This degree of temperature control could withstand both high and low external temperatures. Figure 5 shows that the cells could be held quiescent by chilling for at least 28 days without adversely affecting their subsequent viability and development. Moreover, it is seen in Fig. 6 that the cells as prepared in their dishes also could survive the simulated hazards of lift-off and recovery.

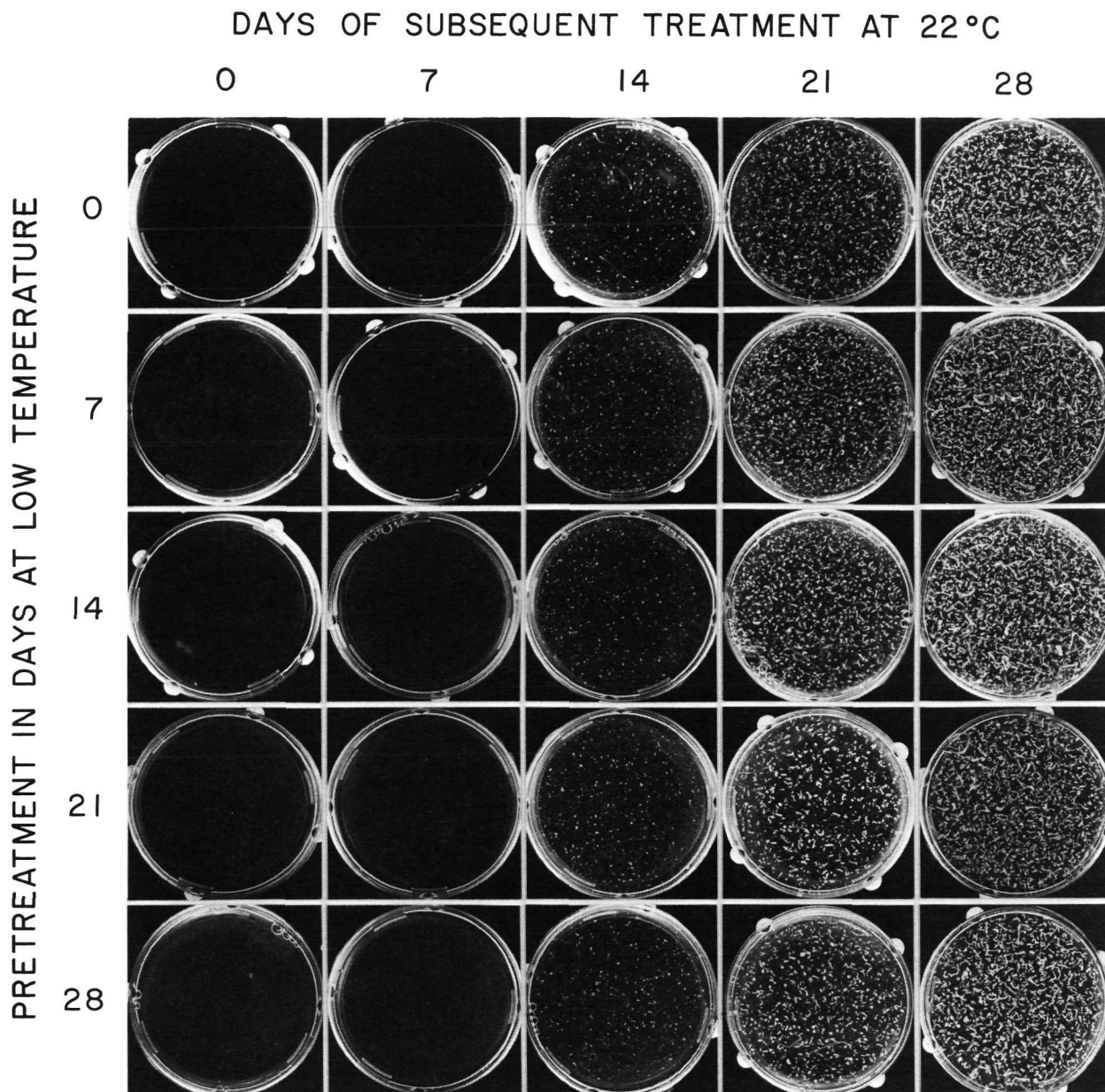


Fig. 5. Shows that morphogenesis in totipotent cells of carrot is depressed by low temperature (4°C) and this is subsequently reversed at higher temperature (22°). The growth shown on each dish was the outcome of the number of days at 4°C, as indicated on the vertical scale at the left, followed by the number of days at 22°C shown on the horizontal scale. Note that a maintained low temperature for as long as 28 days, during which growth did not occur, did not interfere with the ability of the cells to develop when they were transferred to the higher temperature.

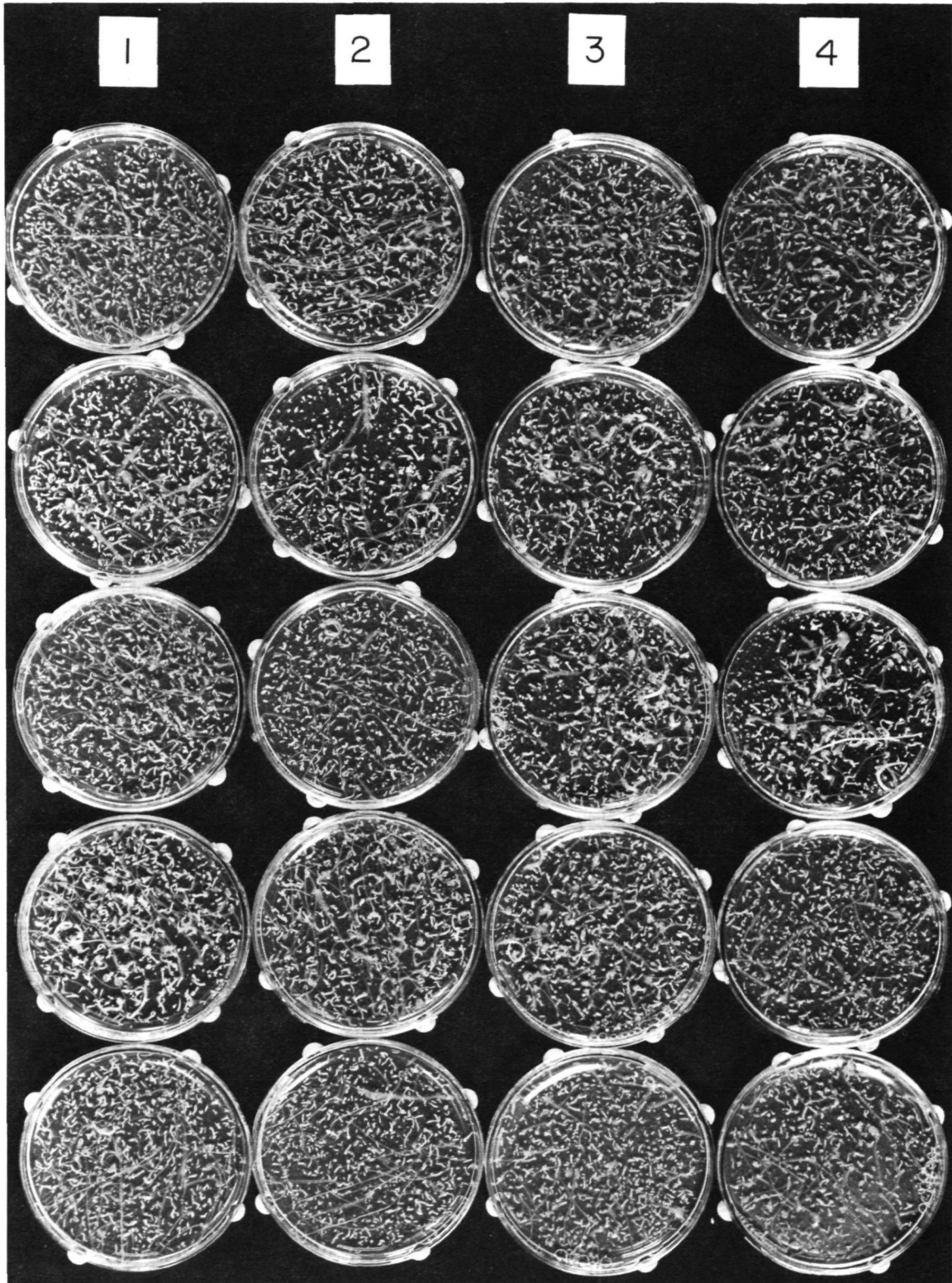


FIG. 6. Organized growth from totipotent cells of carrot after tests to determine the acceptability of the system for flight and the biocompatibility of the hardware.

From 9 replicate dishes in each batch subjected to a treatment, 5 representative ones were selected for photography to show the growth that finally occurred. (Fig. 6)

Row 1 represents dishes that served as controls at Ames Research Center.

Row 2 represents dishes that were exposed at Ames Research Center to conditions simulating the anticipated mechanical and temperature stresses that the cultures were expected to undergo upon lift-off, flight and retrieval. (For details see a report entitled "1975 Joint U.S./U.S.S.R. Biological Satellite Mission: Project Development and Support" by John Tremor, et al.)

Rows 3 and 4 represent comparable materials which remained at Stony Brook. The dishes in each of these rows were subjected to treatments which, in terms of time and temperature only, could simulate those during flight and recovery, namely:

5°C for 3 days, representing pre-flight chilling;

22°C for 21 days, representing the period of flight;

53°C for 3 hours, representing the re-entry from orbit;

5°C for 4 days, representing the post-flight chilling in transit.

The visible results in this typical set of tests (one of several) showed that:

- a) the responses of replicate dishes in a batch and of replicate batches were quite uniform.
- b) the cells in the dishes retained their viability after the full mechanical and temperature stresses of the experimental plan.

vi) Distribution of Canisters

Canisters KF-1 through 10 were packed in a Biotransporter and flown to San Francisco, California on November 20, 1975 (transport was via United Airlines flight 25 which arrived in San Francisco at 2:30 P.M. Pacific Standard Time). Upon arrival at ARC by surface transportation, the experimental canisters were transferred to a 4°C incubator. They were re-packed in 2 Biotransporters on November 21 and were taken to Moscow by Dr. John W. Tremor of the Flight Experiments Office at ARC. Table 1 shows the final distribution of experimental canisters. On the morning of November 25 the canisters were removed by Soviet personnel at the launch site from the Biotransporter at about 9:10 to 9:30 and loaded into the actual flight containers (Fig. 7). This flight "hardware" was shared with the *Fundulus* experiment, designed to study the effects of weightlessness on the development of sensory systems in *Fundulus heteroclitus* starting with roe at various stages of development. From the time at which loading was completed until the launch, the flight containers were kept at ambient room temperature.

A unique feature of Cosmos 782 was the presence of a centrifuge aboard the spacecraft. The flight container with the canister of the carrot cell culture experiment (KF-2) was situated such that there was an artificial gravitational force of 1 g. The position of the flight container on the centrifuge is shown schematically in Fig. 8 as number 2. The flight canister (KF-1) that was to be subjected to zero

Table 1. Allocation of Experimental Canisters for Cosmos 782 Experiment.

				Experimental Canister Number
U.S.S.R.				
Flight-0 G	1 flight container	1 unit	KF-	<u>1</u>
Flight-1 G	1 flight container	1 unit	KF-	<u>2</u>
Synchronous Stationary	1 flight container	1 unit	KF-	<u>5</u>
Synchronous Centrifuge	1 flight container	1 unit	KF-	<u>6</u>
Back-up-0 G	1 flight container	1 unit	KF-	<u>3</u>
Back-up-1 G	1 flight container	1 unit	KF-	<u>4</u>
Total	6 flight containers	6 units		
Ames Research Center Controls				
Stationary				
Horizontal	1 flight container	1 unit	KF-	<u>9</u>
Vertical	1 flight container	1 unit	KF-	<u>7</u>
Clinostat				
Horizontal	1 flight container	1 unit	KF-	<u>10</u>
Vertical	1 flight container	1 unit	KF-	<u>8</u>
Total	4 flight containers	4 units		
Stony Brook Controls				
Stationary				
Horizontal	1 flight container	1 unit	KF-	<u>13</u>
Vertical	1 flight container	1 unit	KF-	<u>14</u>
Clinostat				
Horizontal	1 flight container	1 unit	KF-	<u>11</u>
Vertical	1 flight container	1 unit	KF-	<u>12</u>
Total	4 flight containers	4 units		

CARROT CELL CULTURE FLIGHT HARDWARE
CONFIGURATION (K-102), WITH EXPERIMENT K-104

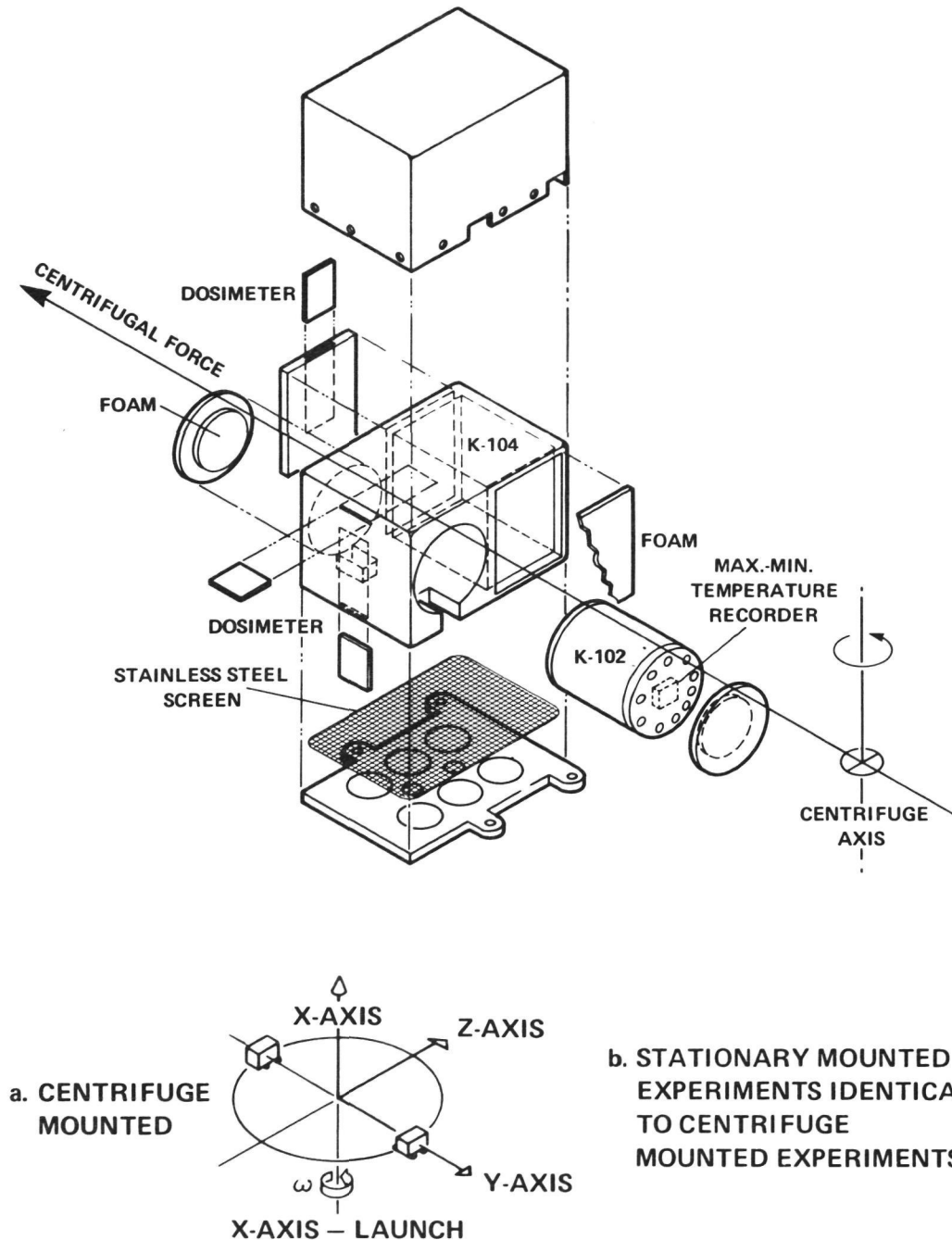


Fig. 7. Diagrammatic representation of a flight canister (cf. Fig. 4) and its orientation in a flight container. Note the extensive foam padding and the fact that the canister has direct access to ambient air via openings in the base plate. The scheme also shows the axis of centrifugation when mounted on the centrifuge and that there was a similar stationary flight container with its canister in the stationary mounted position.

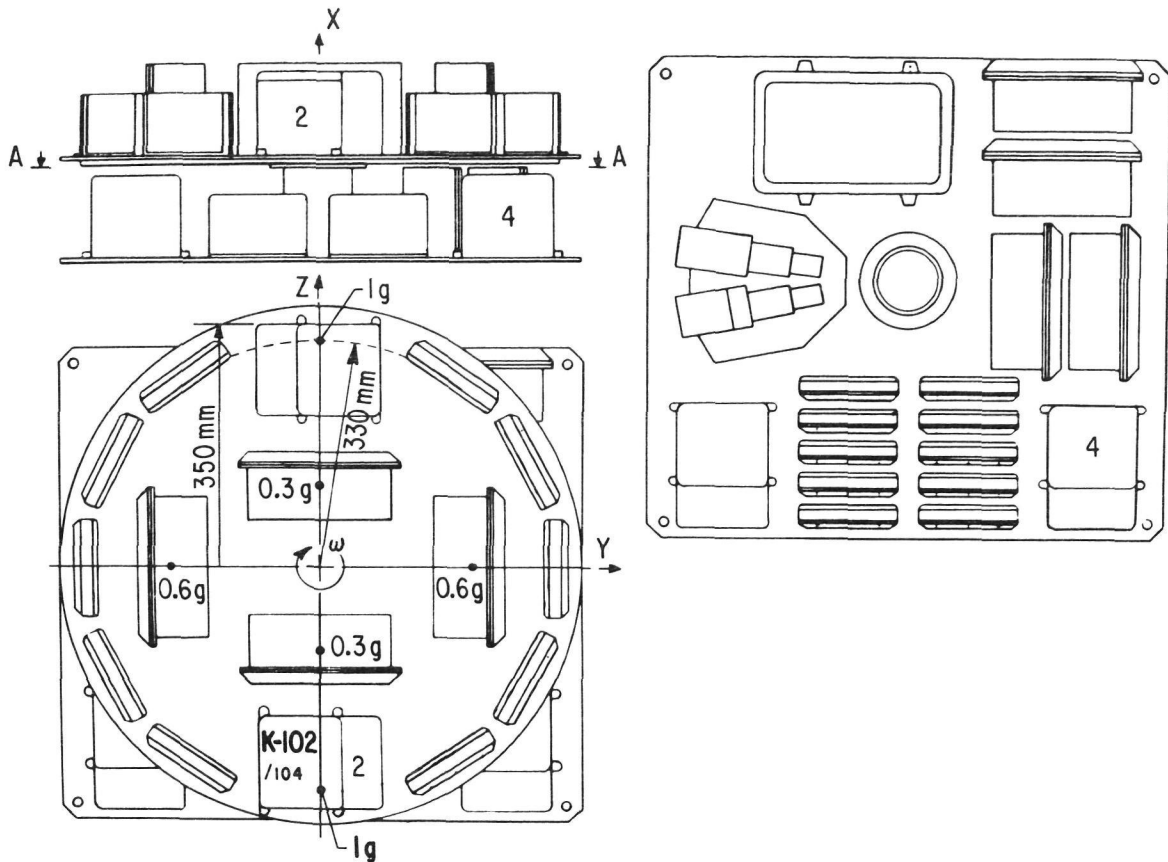


Fig. 8. Diagram of the centrifuge aboard Cosmos 782. This is shown in profile (upper left), where X represents the axis of rotation of the centrifuge, 2 represents the container which holds the canister of 9 petri dishes at 1 g., and 4 represents the similar stationary container which holds the canister of 9 petri dishes at 0 g. The diagram at the lower left shows the rotating platform with the location of this carrot cell experiment as housed in the container 2 in the form of 9 petri dishes in a flight canister. This location provided 1 g conditions and although other locations noted gave other gravitational stimuli, these were not available for use in the experiment. The diagram at the upper left shows in surface view the stationary platform and shows the container 4 with its carrot cultures.

gravity (0 G) was maintained in a stationary position just beneath the rotating part of the centrifuge (Fig. 8, number 4). Launch was achieved on November 25 at 8:00 P.M. Moscow time (12:00 noon EST in New York). The centrifuge was started only "minutes after launch" and was stopped on December 15, about 300 minutes before re-entry.

vii) Ground Controls

The canisters serving as ground controls were removed from 4°C refrigeration on November 30 at 3:00 P.M. EST (12:00 noon PST) and placed into their appropriate environmental situations (cf. Table 1). The experimental plan demanded that these be maintained under the prescribed conditions for a period of time identical to actual flight time (i.e. 19.5 days). At the end of the necessary period for the ground controls being performed in California the canisters were placed in a 4°C ± 2 Biotransporter and shipped to Stony Brook on the same day and accompanied by Mr. Robert Mah of NASA, Ames Research Center.

viii) Retrieval of flight material. The space capsule landed and was retrieved at 9:00 A.M., Moscow time on December 15, 1975. The first recovery team reached the space craft in about 23 minutes and the satellite was covered with a "mobile laboratory" tent within an additional 52 minutes. The carrot cell culture material was unloaded at 340 minutes after landing of the spacecraft. The flight canisters were transferred to a Biotransporter. Flight canisters KF-1, 2 and 3 (cf. Table 1) were made available for examination to A.D. Krikorian (A.D.K.) on Wednesday, December 17, 1975 at the Timiriazev Institute of Plant Physiology, Moscow around 11:00 A.M. The canisters were

opened by A.D.K. in the presence of Raisa G. Butenko (R.G.B.) and Natascha Dimitriaeva (N.D.) in a "clean room" utilized as a transfer facility for plant cell culture work. The plates were examined visually but not opened. By mutual agreement, Dishes 3, 7 and 9 of each canister were allocated to the Soviet investigators. Dishes KF 1-4, KF 2-5 and KF 3-4 were photographed and then fixed with glutaraldehyde for our use (see Appendix A for exact details of the fixation). Dishes KF 1-3, 1-7 and 1-9; KF 2-3, 2-7 and 2-9 and KF 3-3, 3-7 and 3-9 were photographed by A.D.K. and fixed by Dr. V. Ongko. (Dishes #3 were to be glutaraldehyde treated whereas dishes 7 and 9 were fixed in Brodskii and Carnoy's fluids respectively). (Brodskii-Formalin (4%) 10 parts; ethanol 96% 3 parts, glacial acetic acid 1 part. Carnoy's-glacial acetic acid 1 part, chloroform 3 parts, ethanol 96% 6 parts.) The fixed material for use in the U.S.A. was retained by A.D.K. for subsequent transit from Moscow to the U.S.A. All other canisters after removal of dishes released to the Soviets were prepared for transport to the U.S.A. by replacing the dishes removed with "blank" plastic petri dishes. Canisters KF-1, 2 and 3 were taken to New York by Dr. David Feller on December 21, 1975. They were accepted by F.R. Dutcher (F.R.D.) at 12:00 midnight and were transported to Stony Brook at 4°C and placed in a 4°C incubator until they were examined.

On Monday, December 22, 1975, KF-5 and 6 were made available at the Timiriazev Institute for examination by A.D.K., R.G.B. and N.D. However, the "synchronous stationary ground control" (KF-5) and the "synchronous centrifuge

ground control" (KF-6) canisters were both total losses. For some unknown reason the dishes of KF-6 when recovered and examined had lost virtually all their contents and the interior of the canister was grossly contaminated. However, the plastic dishes when examined showed no cracks or breaks and the lids (strangely) were still in place. The pyrell foam insert was completely overgrown with microorganisms, especially fungi. The "microsil standoff" when examined showed minute "eruptions."

All this leads to the view that these canisters may have been exposed to some extreme conditions of temperature and/or pressure which were not part of the experimental plan. The KF-5 canister retained more of its material within the plates but it appeared that the agar medium etc., had "shifted" -- as if subjected to a centrifugal force. This is all the more curious since KF-5 was to have been kept stationary. In this canister the pyrell foam insert was missing but the microsil standoff showed none of the minute eruptions described above. Again, even though this canister was contaminated, this was not as extreme as in KF-6.

Although 1/3 of the experimental dishes were allocated to Madame Butenko as arranged, we agreed that no useful data or observations could be obtained from KF-5 or KF-6. Moreover, the second "back-up" canister (KF-4) was not made available since its precise whereabouts seemed not to be known. On December 23, 1975 A.D.K. returned to New York with canisters KF-5 and 6. Moreover, he brought back the fixed dishes KF 1-4, KF 2-4 and KF 3-4. On December 24, dishes 3, 7 and 9 of canisters KF-7 through 14 inclusive

(cf. Table 1) were fixed according to the Soviet Union investigators' fixation schedule. They were then carefully packed and shipped via Biotransporter direct from J.F. Kennedy Airport in New York to Moscow on December 27, 1975 and its safe arrival was acknowledged.

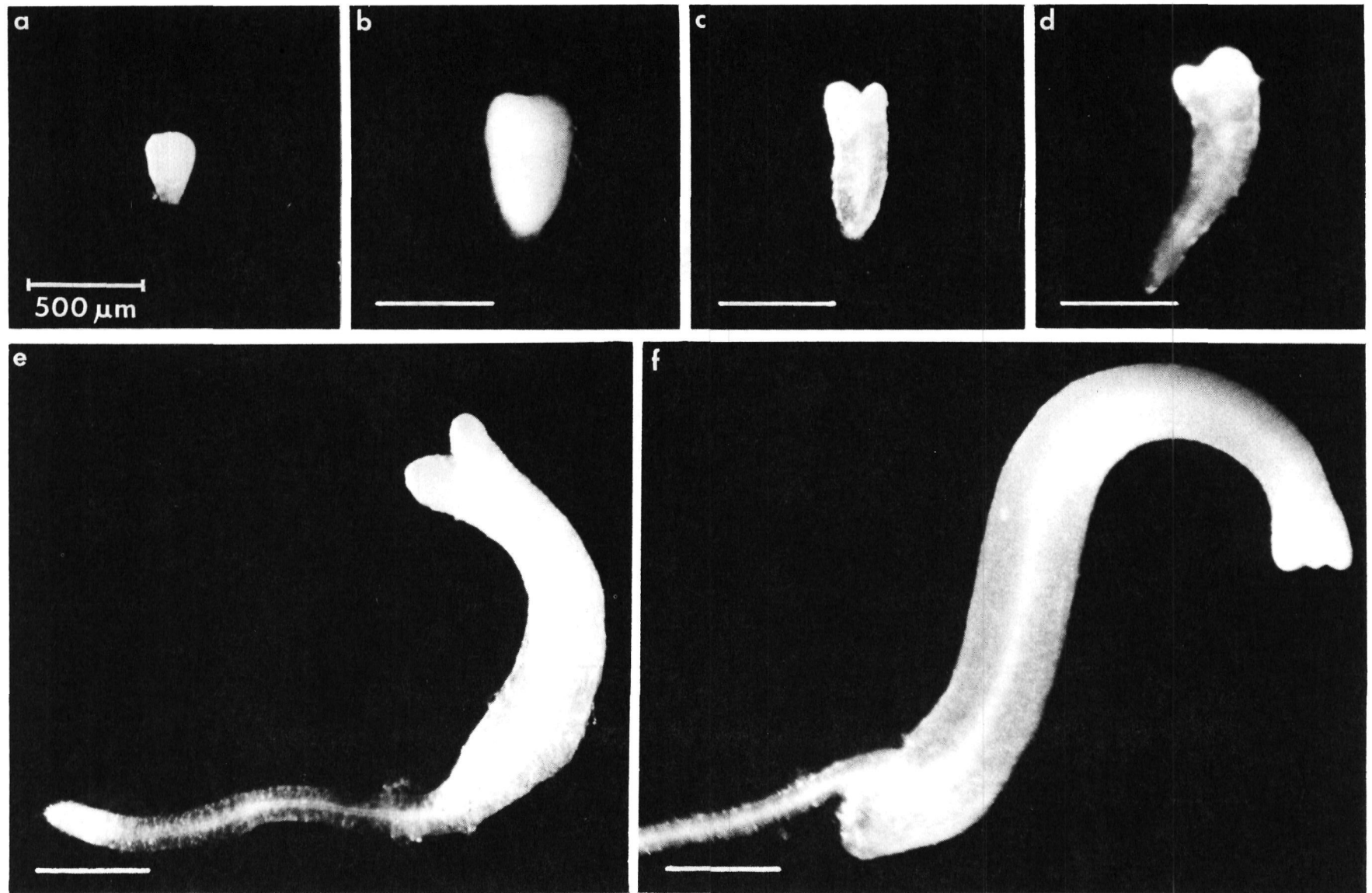
(ix) Counting and scoring of organized Forms. "All or none" conclusions, i.e. whether morphogenesis did or did not occur can be drawn merely on visual grounds in this system. Therefore, many of the results are recorded photographically. These illustrations should portray the visible consequences of the treatments and over-ride the minor variations as between replicate dishes or within the crop on any given dish.

On the other hand, it is much more complicated to establish the reality of any apparent qualitative or quantitative differences between treatments. The clone of wild carrot used here presents both advantages and disadvantages in that substantial variability in the expression of morphogenetic potential is encountered. The statistical problems that this entails are outlined below.

The size of the flight containers allocated to this experiment only accommodated 9 replicate plastic petri dishes per canister (cf. Fig. 4). Since the number of cellular units on any given dish is large, the numbers available for observation permit statistically significant differences to be detected, if any exist.

The possibilities of organized vs. non-organized forms range from free cells and clusters which remain quiescent, to random unorganized proliferations as in so-called callus masses, through the inception of the organized growing regions

of shoot and/or root apices, to completely formed embryoids and small plantlets. In fact, all of these forms occur. Large numbers of heart-shaped, torpedo and more advanced stages of embryogenesis could be detected. Although the criteria of somatic embryogenesis do not yet readily lend themselves to rigorously quantitative data, arbitrary scorings were made using conventional embryological designations. The variety of growth forms observed was separated into four sequential stages of embryonic growth as follows: stage 1 consists of heart-shaped forms, (cf. Fig. 9a); stage 2 consists of torpedo-shaped structures, these units varied in size but were generally less than .75 mm in overall length (cf. Fig. 9b and c); stage 3 consists of advanced embryonic forms with a very distinct root and were generally between .75 and 1.5 mm in overall length (Fig. 9d); units in stage 4 were really small plantlets, each with a well developed root and generally were longer than 1.5 mm in overall length. These forms had very immature shoots (cf. Fig. 9e and f). The number of smaller units, such as those at stages 1 and 2, were determined by recording the distinct forms as seen under a dissecting microscope in three separate 1 cm circles taken at random and by multiplying this value by an appropriate factor (9.375) to obtain the total number present per dish. The smaller numbers of larger and more advanced forms (as at stages 3 and 4) were also scored and counted by scanning the entire dish systematically. Dishes numbered 1, 2, 5, 6, and 8 of each canister were scored and counted as described; dishes numbered 3, 7 and 9 were allocated to the Soviet investigators and thus were not counted by us. Dishes numbered 4 were fixed but not counted.



FIGS. 9 a-f. Organized structures grown from cultured cells of wild carrot. These embryonic growth forms were arbitrarily scored for purposes of counting. Fig. 9a represents a heart-shaped embryoid; b and c show two structures -- the first is a so-called very early stage 2, that is an early torpedo-like embryonic structure, the second is a late torpedo (also stage 2); Fig. 9d represents a still later growth form (stage 3) showing a distinct root but abbreviated shoot; Figs. 9e and f show 2 forms categorized as stage 4. The one shows a well-developed root and a clearly recognizable shoot, the other shows a poorly developed shoot but with a very long root. The root is so long that only a portion may be seen.

(x) Light, transmission and scanning electron microscopy procedures. Materials were fixed using the detailed schedules presented in Appendix A at the back of this report. This fixation served for the light, transmission electron and scanning electron microscopy procedures and was recommended to us by Dr. Myron C. Ledbetter, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York.

Materials were further prepared for light microscopic examination by the methods of Feder and O'Brien (1968). Somatic embryos were cleared using several techniques, especially that of Debenham (1939).

Transmission electron microscopy (TEM) procedures were those used routinely by Ledbetter at Brookhaven. Embedding was according to the method of Luft (1961).

Scanning electron microscopy (SEM) was carried out on somatic embryos in the following way. The embryos, free as possible of agar, were washed in buffer (see Appendix) and treated with osmium and thiocarbohydrazide in the "OTO" (osmium-thiocarbohydrazide-osmium) method of Kelley et al., (1973). They were dehydrated step-wise in a graded series of acetone-water mixtures and dried by Anderson's (1951) critical point method using liquid carbon dioxide. The dried specimens were electrically conductive from the OTO treatment and were observed in the SEM either uncoated or coated with gold at accelerating voltages from 1 to 30 kV.

Both TEM and SEM examination was carried out at Brookhaven National Laboratory by Dr. Myron C. Ledbetter.

(xi) Procedures used to assess the subsequent viability and growth responses of embryonic forms that developed in space and in ground controls.

(a) Growth of Embryos into plants

Embryos ranging in their development from stages 1 through 4 inclusive were removed from dishes under aseptic conditions with fine forceps and the aid of a dissecting microscope. (The larger forms were removed first.) The somatic embryos were transferred to 50 mm diameter petri dishes onto a medium of B_{MS} salts, vitamins, inositol and 3% sucrose solidified with 1% agar. Prior to "planting" the embryos, however, they were "washed" by streaking them through a basal medium (B_{MS} + inositol thickened with .5% agar). Three or four embryos at the same stage of development so treated were evenly distributed on the dishes, and placed in darkness at 21°C in an environmentally controlled growth chamber. After approximately 1 month of growth in continuous darkness (except for brief periods of examination in the light, the then well-developed plantlets were removed from the small petri dishes and were re-planted individually into large test-tubes (approximately 37 x 215 mm, containing ca. 50 ml of medium) on a 1/2 strength B_{MS} + vitamins + .5% sucrose medium with a slightly softer agar (.6%) and the growth was allowed to continue in diffuse continuous light. After another month or so, the plantlets were removed from the tubes for transferral to a soil mixture in pots. The roots were gently washed in luke-warm tap water under a running faucet to free them completely of agar medium. Individual plantlets were placed carefully in a pre-sterilized mixture of equal parts sand and "Jiffy-Mix" in 2 inch pots.

(It was essential in this early period to guard against fungal contamination and desiccation). After a week or so under misted propagation bench conditions in the greenhouse, the plants were vigorous enough to withstand a normal open-bench greenhouse environment. At the time of this writing, the plantlets have been transplanted to 4 inch pots after additional growth of 2 months.

(b) Visual and photographic recording of growth

Periodic visual examination of the plants so grown was made and photographs taken.

The Realization of the Experimental Plan

The detailed experimental methods as outlined were followed except as the plan in operation encountered unexpected complications. The entire loss of the synchronous ground controls in the Soviet Union (KF-4, cf. Table 1), which could have served as a further control to detect any possible hazards encountered in transit to the U.S.S.R., have already been noted. For reasons that will be apparent later, these losses will not seriously affect the conclusions that will be drawn.

The hope was that despite the large number of separate petri dishes and canisters, and the complicated operations, the entire experiment would be carried out under completely aseptic conditions. This hope was realized in that the units that grew on the petri dishes, and which could be removed from them by means of forceps, were aseptic even in their subsequent growth on fresh media. However, before the flight of Cosmos 782 was completed, a very slow-growing bacterial contaminant was detected, sparsely, on some of the dishes retained at Stony Brook as additional

stationary ground controls, and as clinostat controls. Under observation, this contaminant grew very slowly and did not overgrow the carrot colonies, so that the cultures could easily be observed, counted and removed aseptically for further growth. Therefore, the examination of the cultured units on the plates at the end of the flight period could be, and was, completed as though the contaminant did not exist.

Observations and Results

The Cosmos-782 biological satellite was in flight from November 25 to December 15, 1975. Fig. 10 shows the sequence of operations starting from the actual preparation of the cells at Stony Brook, their transmittal to Ames Research Center, Moffett Field, California, their transferral to Moscow and eventually to the Soviet launch site. The date of launch, the flight period, retrieval and final disposition in the Soviet Union and in the U.S.A. are also shown. A complete schedule of the operations as performed, with special reference to temperature, is included as Appendix B to this report.

The crucial feature of this diagram and the detailed time schedule of preparation, transmittal, flight and "post-flight" operations as provided in Appendix B is that at no time after the final preparation of the cells until their transmittal to the launch site in the Soviet Union were the cells at a temperature at which vigorous metabolic activities could resume and cell division proceed. A close examination of the schedule reveals, however, that there was a period of 14 1/2 hours just prior to launch during which the cells could slowly approach the unspecified

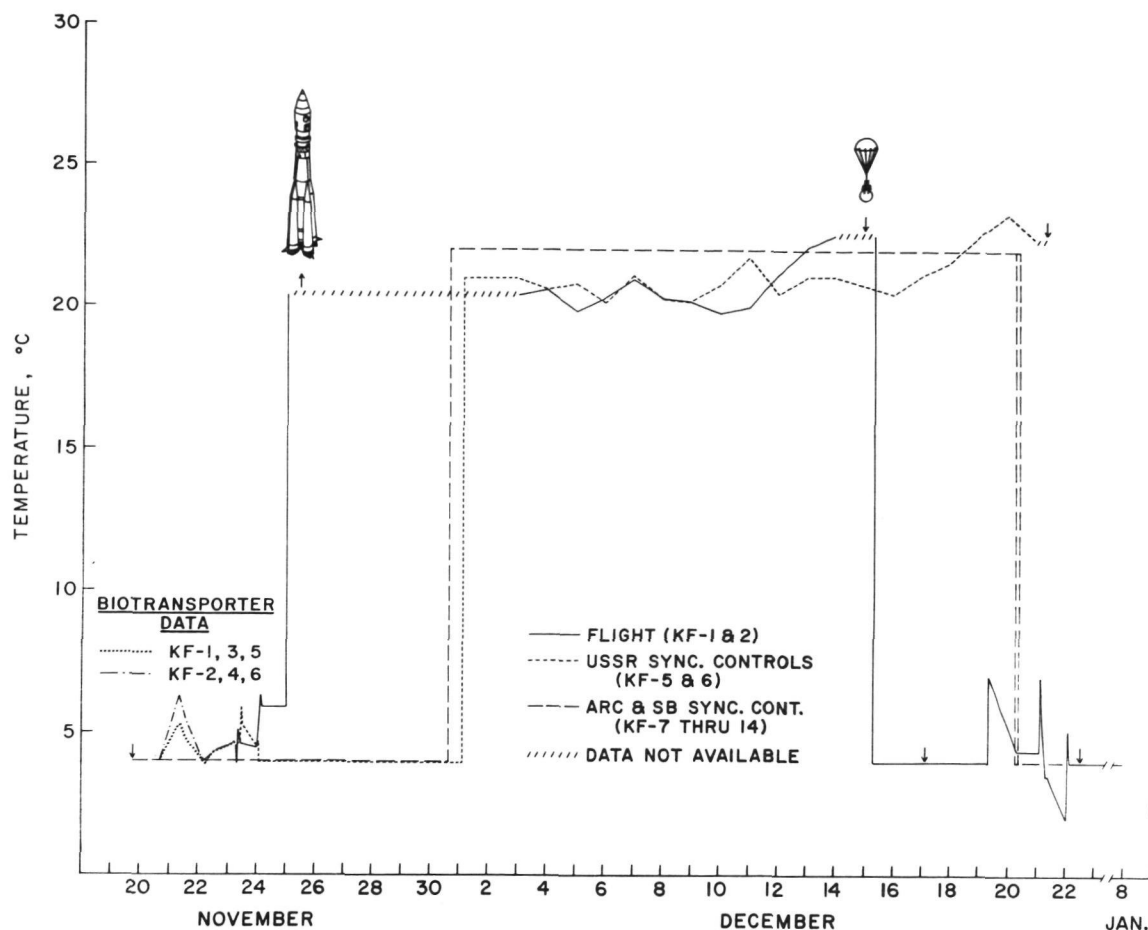


Fig. 10. Shows diagrammatically the time course of events with special emphasis on the temperature of the canisters throughout the entire experiment. The first small arrow ↓ at Nov. 19, represents the actual date of preparation of the cells. The next arrow ↑ (Nov. 25) represents the "lift-off" of the flight. The third arrow ↓ represents "landing" (Dec. 15) and the fourth ↓ the actual delivery of the flight materials at the Timiriazev Institute of Plant Physiology in Moscow. The arrow ↓ at Dec. 21 represents the delivery of the synchronous ground controls (KF-5 and 6) to the Timiriazev Institute. The very last arrow ↓ (at Dec. 22) represents the day when observation began at Stony Brook on material brought from the USSR as well as the "synchronous ground controls" from Ames Research Center and Stony Brook.

"ambient room" temperature. This occurred during the loading of the flight canisters into the flight containers and their installation of the space vehicle until the time of actual "lift-off". Nevertheless, since the cell generation time for carrot cells in agar during their initial growth is of the order of 1 day (cf. Blakely, 1964), there is little chance that any cell division actually occurred in this period. Therefore, the first and critical requirement for a successful space experiment, namely that no cell division or organized development attributed to the period in space could have occurred on earth was met.

It will be apparent from Figures 11 to 15 that morphogenetically competent populations of wild carrot cells in a heterotrophic medium and within a period of 19.5 days in darkness can develop in space into readily recognizable organized forms. For the purpose of this report we will concern ourselves only with the dishes that were actually flown in space. This is appropriate since these are the only treatments that are strictly comparable. The figures are arranged so that ready comparison may be made between 0 g and 1 g dishes which occupied position 1, 3, 4, 7, or 9 in canisters KF-1 and KF-2. Note the numerous embryonic forms and especially the advanced embryos with long roots and conspicuous hypocotyls. The relatively low magnification and the fact that the profuse crop of embryos is dispersed within as well as on agar make it difficult to distinguish all the forms, but typical globular, heart-shaped, torpedo and mature embryonic structures were present. The data presented in Table 2 and Figure 19 were derived from counts made on such dishes.

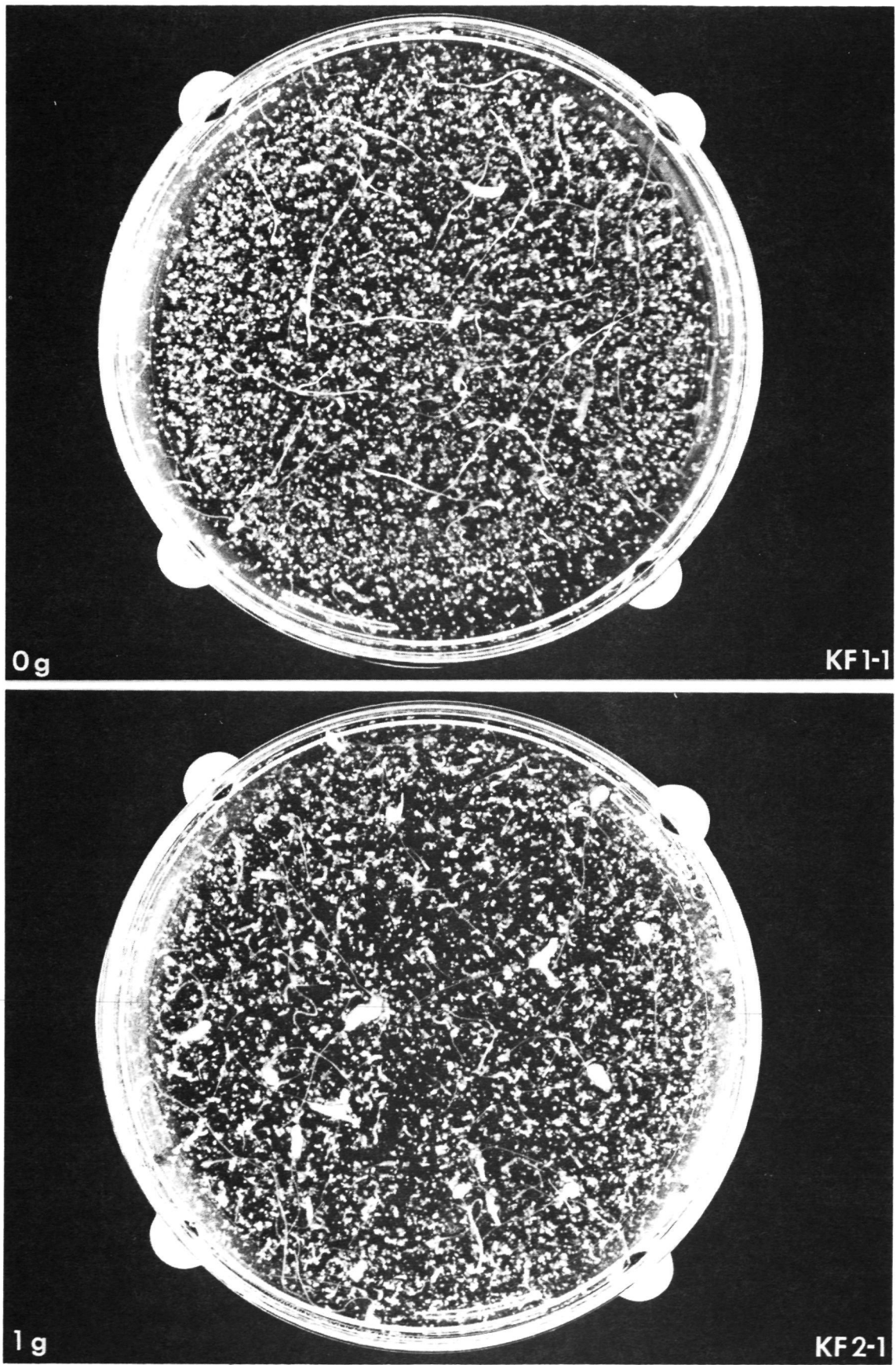


Fig. 11. Growth of carrot cells in position 1 of flight canisters after 19.5 days in space under near-zero g and 1 g conditions.

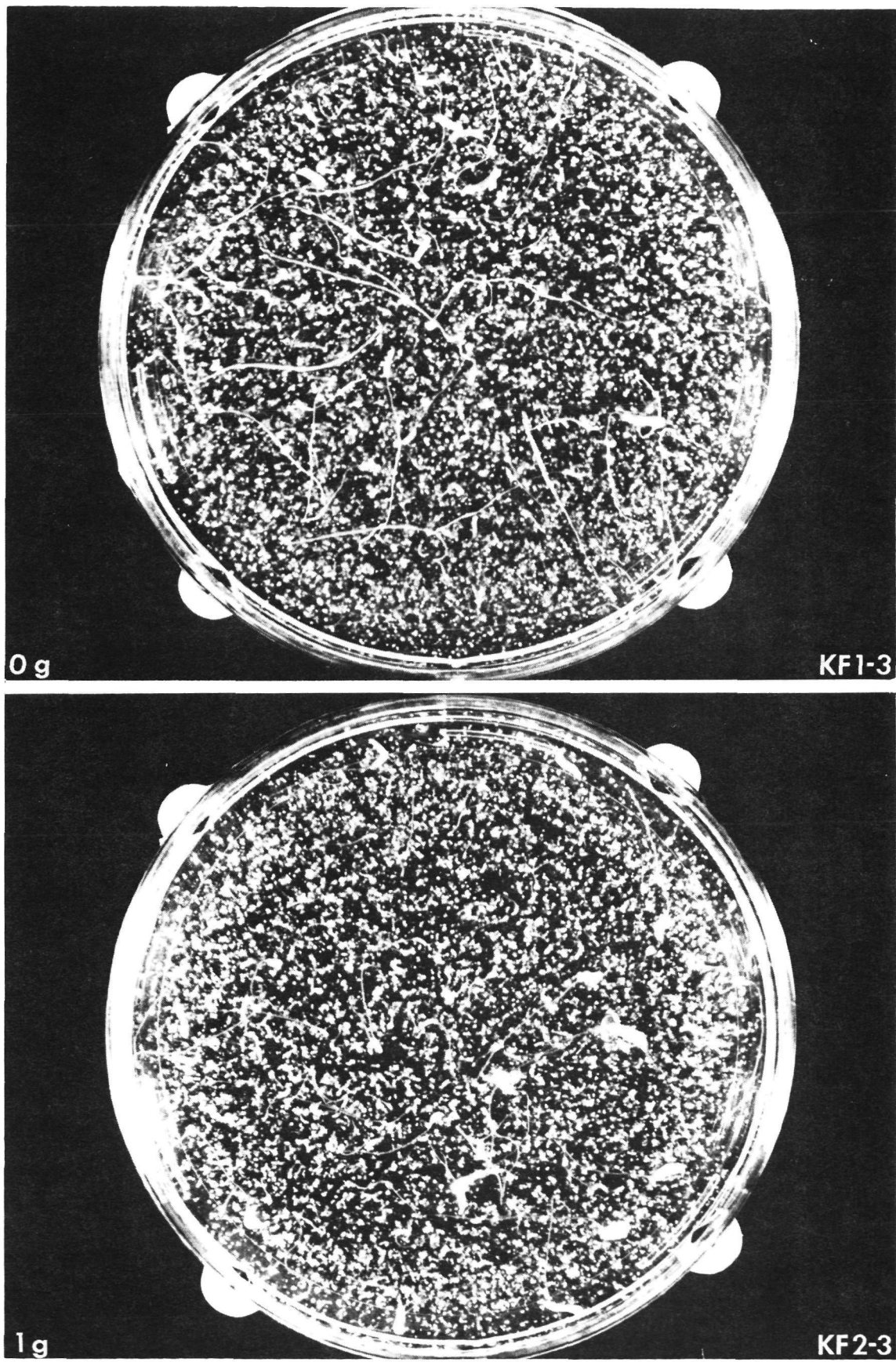


Fig. 12. Growth of carrot cells in position 3 of flight canisters after 19.5 cays in space under near-zero g and 1 g conditions.

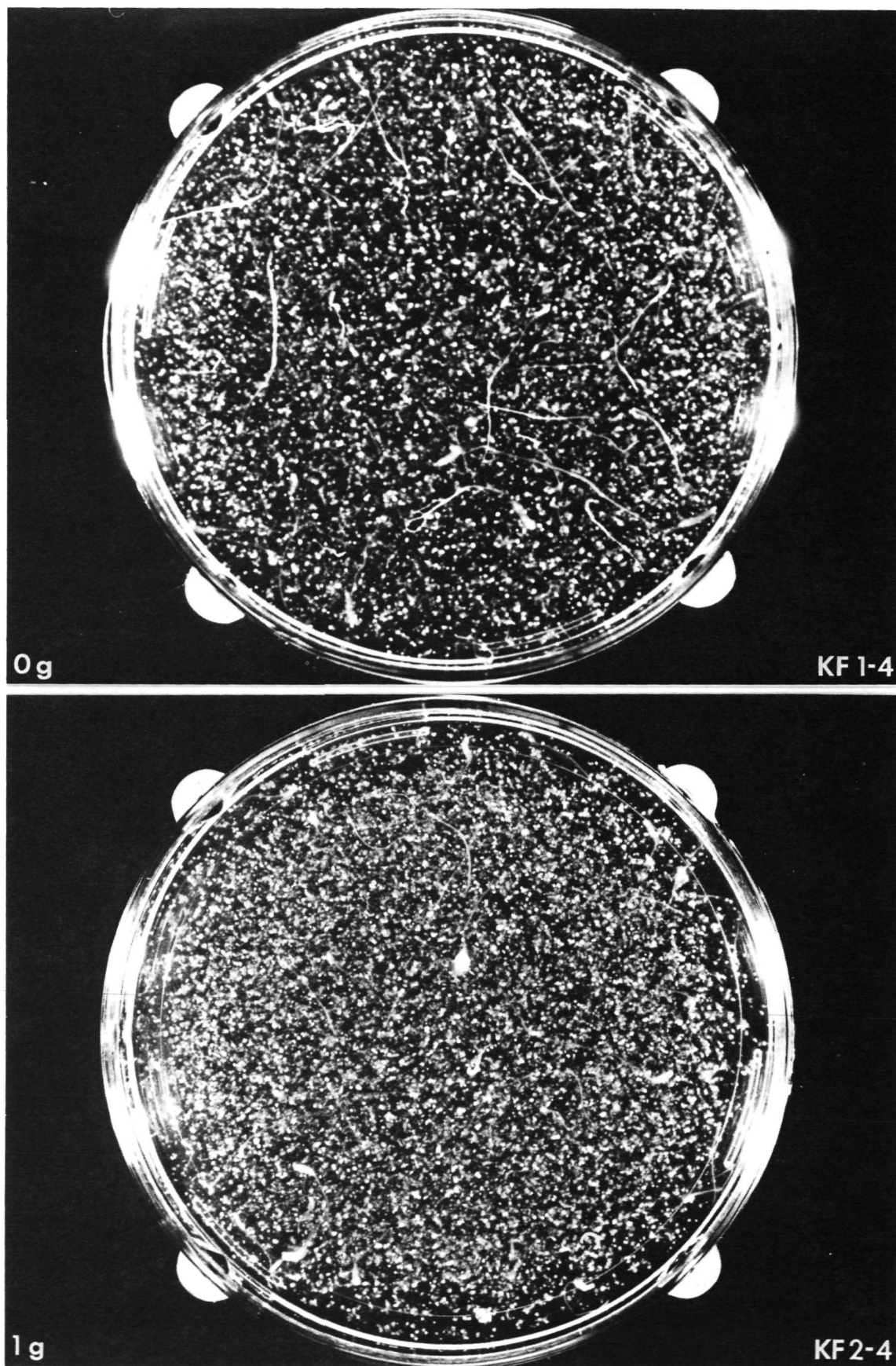


Fig 13. Growth of carrot cells in position 4 of flight canisters after 19.5 days in space under near-zero g and 1 g conditions.

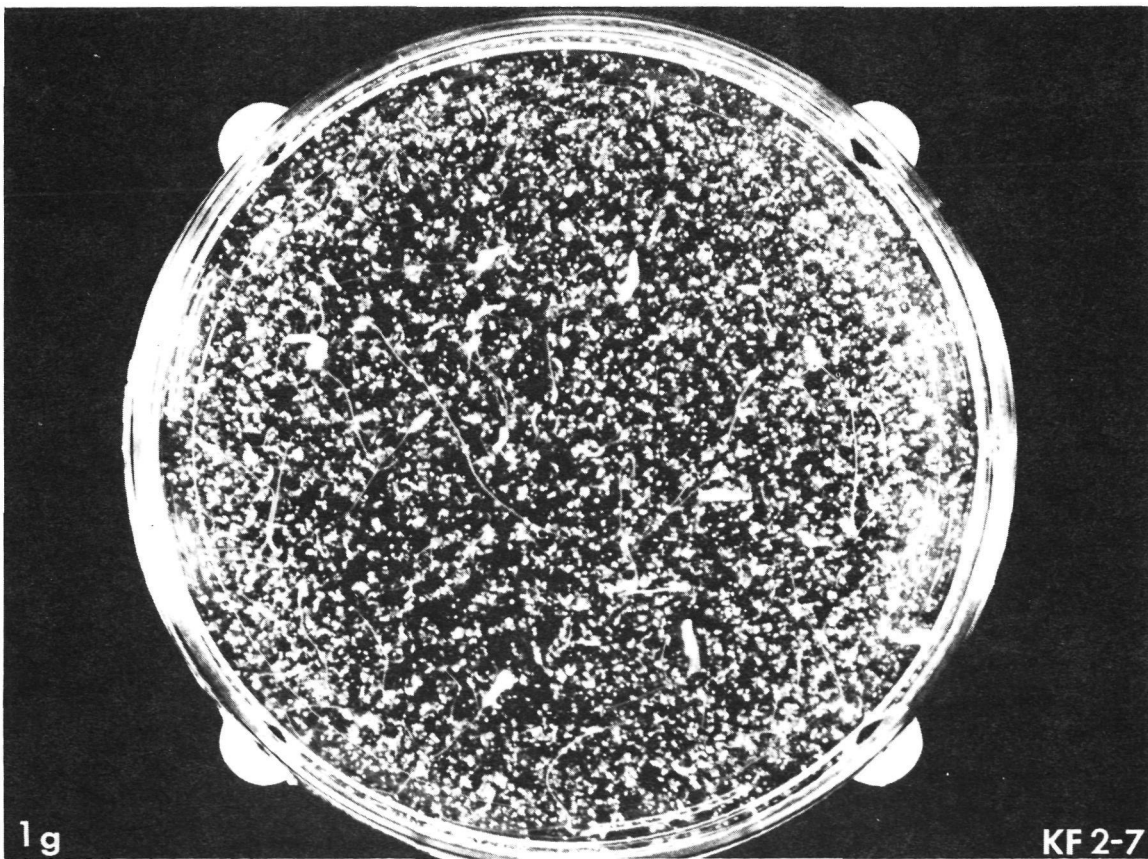
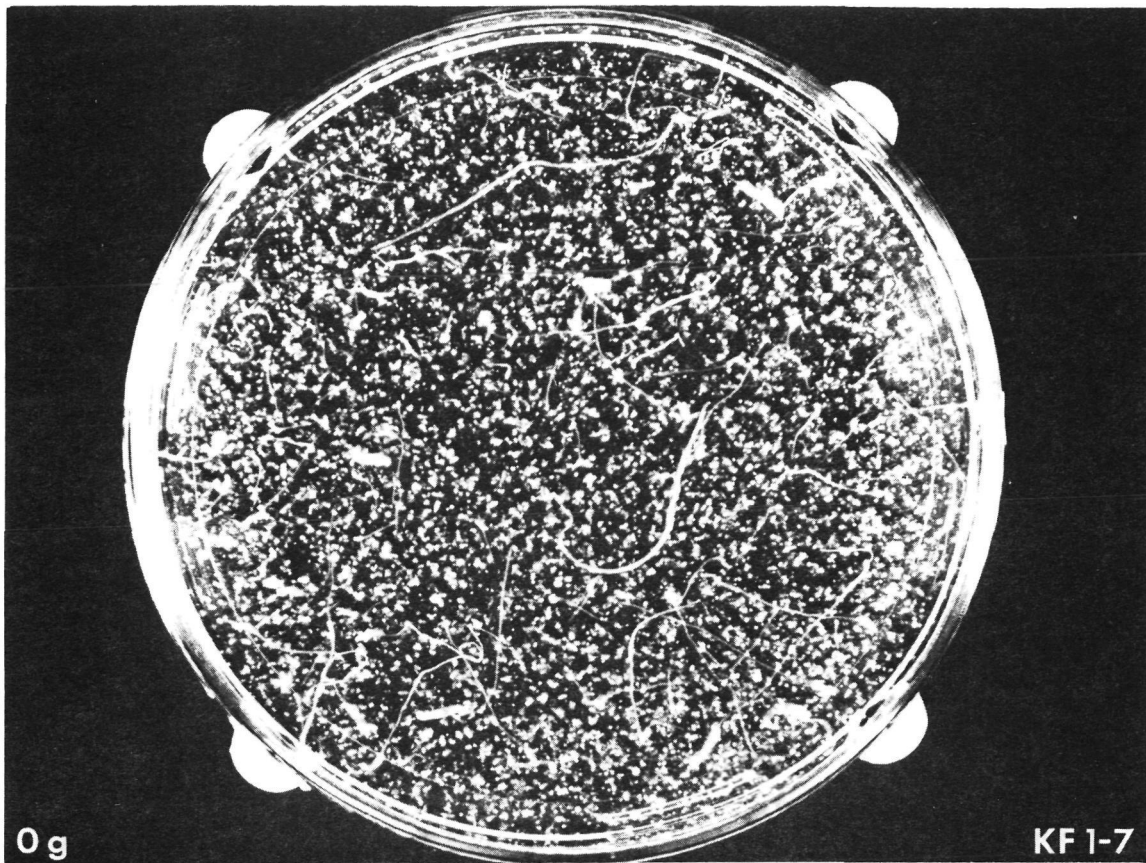


Fig 14. Growth of carrot cells in position 7 of flight canisters after 19.5 days in space under near-zero g and 1 g conditions.

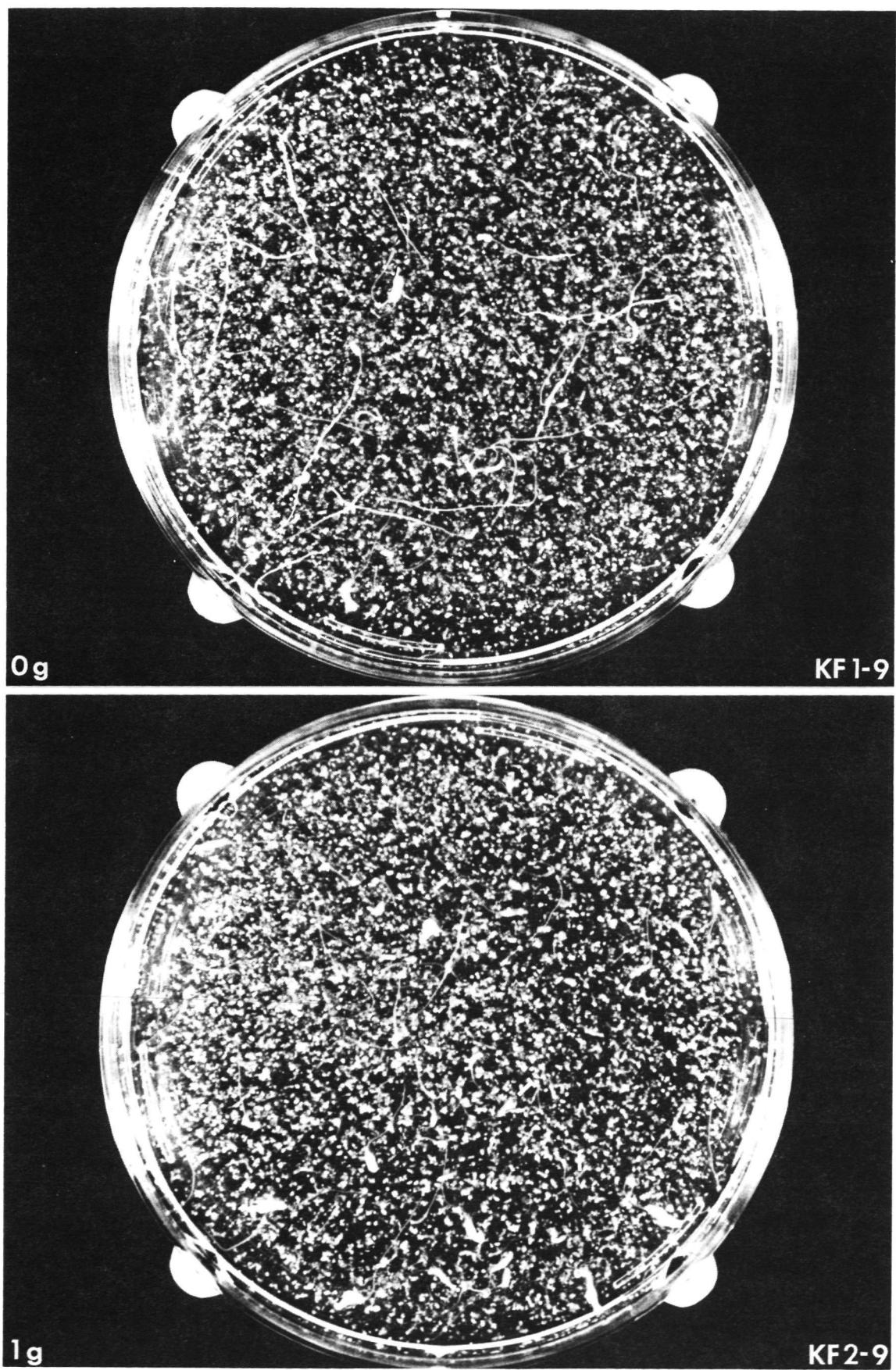


Fig. 15. Growth of carrot cells in position 9 of flight canisters after 19.5 days in space under near-zero g and 1 g conditions.

All forms expected as the cells divided or grew and developed into embryonic forms were encountered on all dishes as flown. These included completely normal embryos and small plantlets, as well as some callus masses derived from random unorganized proliferation of the cells or units dispersed in the culture medium. Moreover, and again as expected, many cells did not divide, for the system, although the most advanced morphogenetically competent angiosperm free cell culture system developed to date, is not yet 100% efficient. Figs. 16 to 18 provide a photographic record of the course of growth of individual carrot units in nutrient agar medium in 50 mm diameter petri dishes. These were obtained after repeated microscopic observations, with an inverted microscope, on totipotent carrot cells as they grew on B_{MS} + inositol and 3% sucrose. Observations were made at low magnification on a large random sample of units plated on agar. Cells or units, chosen at random, were noted as to their position within grids outlined in ink on the bottom of each dish. Cells within these fields were observed and photographed at low magnification (e.g. 4 x objective; 6.0 x ocular) at frequent intervals after inoculation. Because of the relatively low morphogenetic competence of these units, especially at low densities, they were placed at a relatively high density to ensure that a sequence of growth could be observed. Experience indicates the general appearance of the cells that will express their innate totipotency. Unfortunately, it is not easy to follow many individual units from day 1 through all of the remaining stages in a given sequence. Most units are not easily brought into a clear focus; others are obscured by shadows cast by water from condensation on the petri dish; and others which are at early stages in the developmental sequence and appear as though they should yield clear results may be overgrown by adjacent units which were quiescent and non-proliferating at the outset; still other units which one subjectively "predicts" will go on to develop into embryos may cease to develop after only a few cell divisions or do not develop into embryos. Even so, the three representative sequences, showing the same unit throughout at the same magnification as shown in Figs. 16 to 18 respectively confirm earlier observations

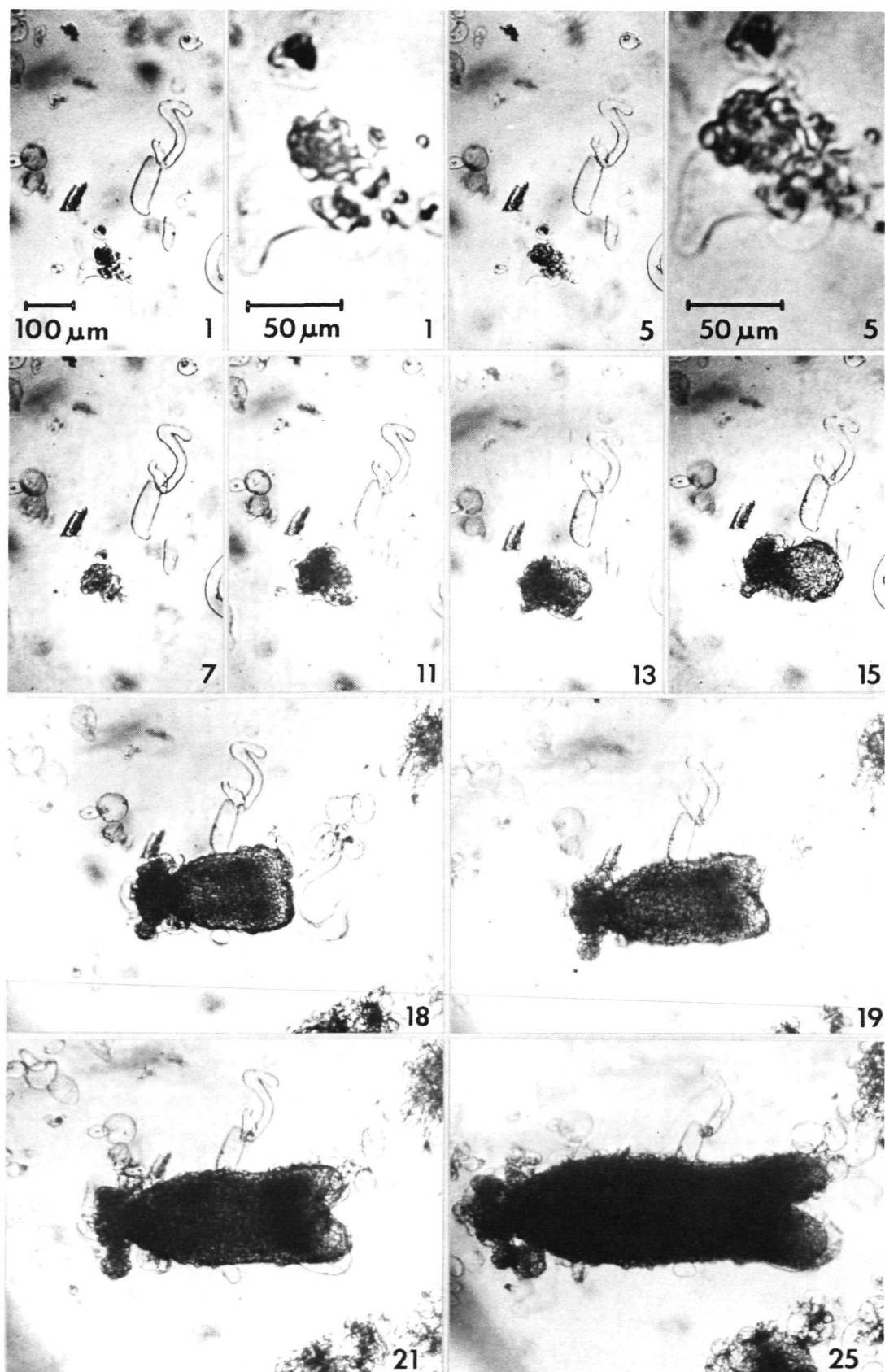


Fig 16. Growth in totipotent cells of wild carrot during somatic embryogenesis. See text for details.

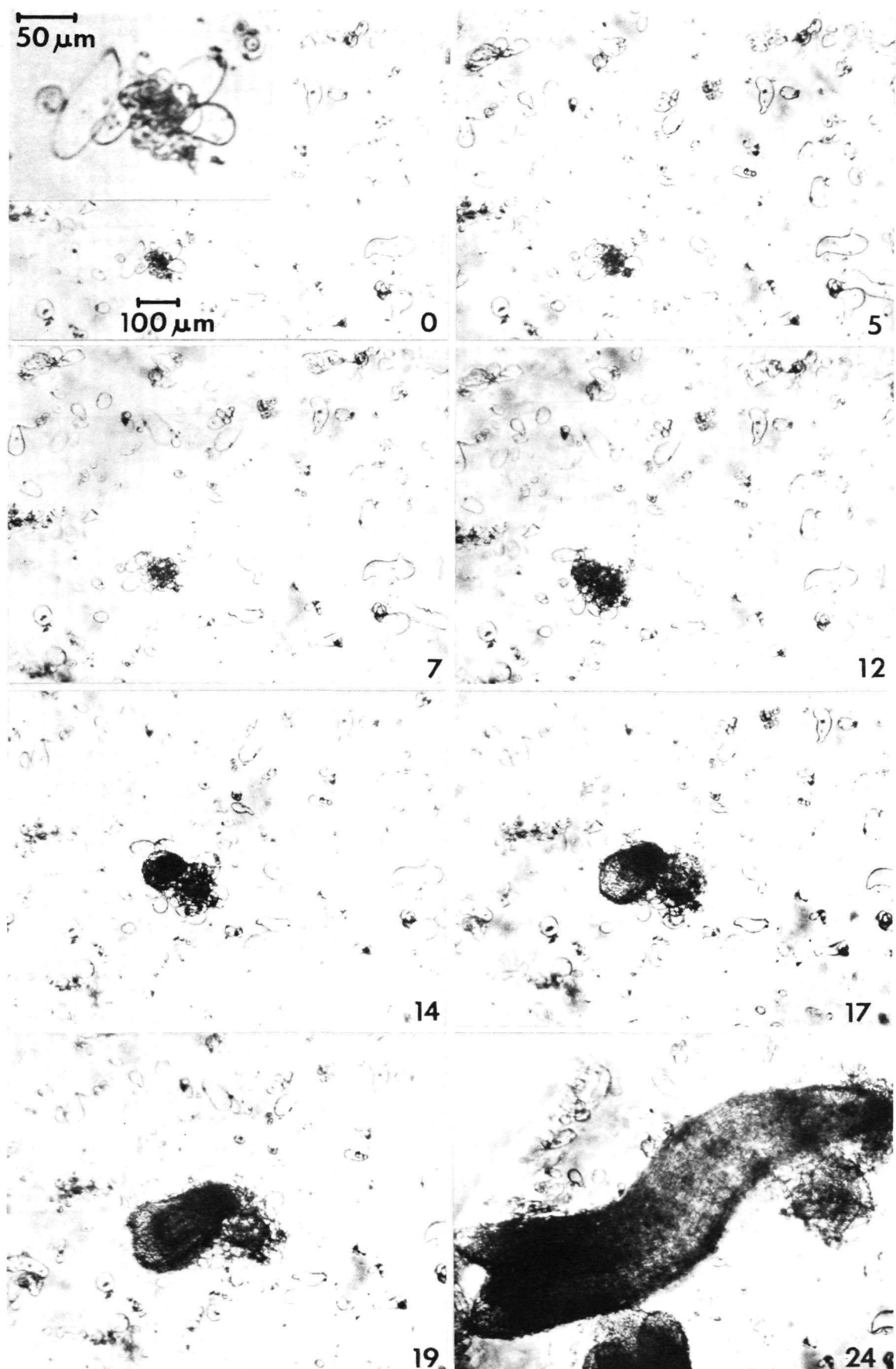


Fig. 17. Growth in totipotent cells of wild carrot during somatic embryogenesis. See text for details.

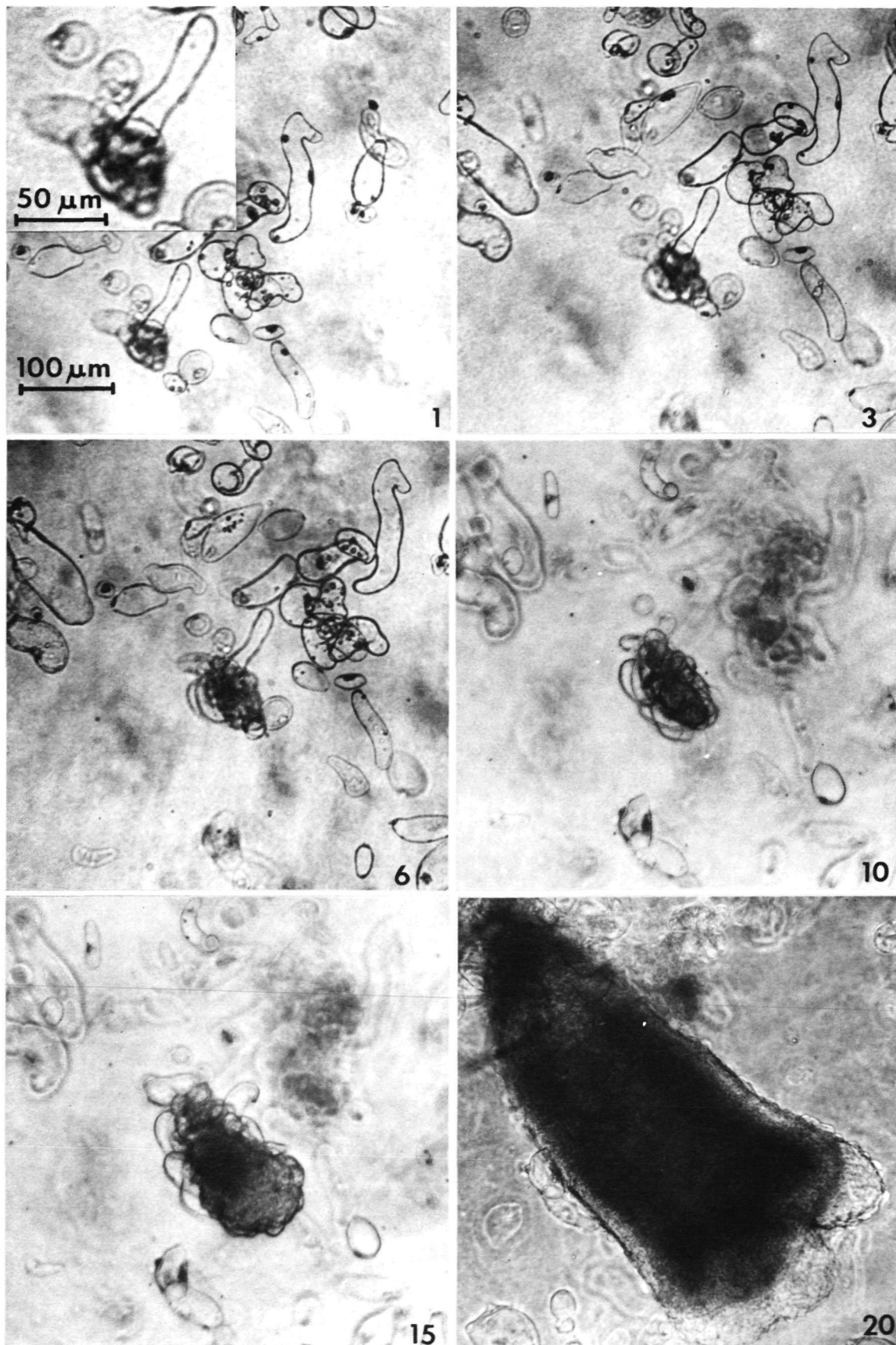


Fig 18. Growth in totipotent cells of wild carrot during somatic embryogenesis. See text for details.

made on carrot cells grown in liquid suspension culture and extend them to the culture conditions in agar as used in this experiment. Each of the three composite figures shows selected stages of development from small units into fully grown embryos. To conserve space the photographic record of all stages, even though available, has not been included. The figures are labeled to show the elapsed time between stages. Moreover, a strict scale of size has been maintained in the printing of the photographs to emphasize the dramatic growth that occurred. A few insets at higher magnification (their magnification indicated by an appropriate scale) are also included. It is particularly noteworthy that a globular pro-embryonic structure develops first and this rapidly forms a heart-shaped embryo. By about 14 days, the critical early events of somatic embryogenesis may be completed and a well-defined torpedo stage embryo emerges. Further development may proceed rather rapidly. In fact, within a period of an additional week, embryos at stage 3 or 4 may arise. It must be emphasized, however, that the figures selected show units developing in seemingly relative isolation. This is so because the photographs have been "cropped" to ease interpretation. Even so, it will be clear by observing cells which are adjacent to the totipotent units, which thus act as "markers", that the units depicted as undergoing morphogenesis were indeed the same ones throughout so that they are shown in sequence as they become progressively more complex. Steward et. al. (1961) pointed out long ago the remarkably faithful recapitulation of normal plant embryogeny (cf. Borthwick, 1931) by these cultured units as grown in liquid in the light.

Not unexpectedly, however, because of the relatively short duration of the flight, the greatest number of organized forms encountered on the petri dishes were tiny heart-shaped embryos (stage 1). Torpedo-shaped embryos (stage 2) were also present but in far fewer numbers. Those present in least profusion were

those designated as very advanced embryonic forms and young plantlets (stage 3 and 4).

Table 2 provides data derived from counts made painstakingly and arduously on plates as to the degree of organization encountered in material from canisters exposed to 0 g and 1 g. (See Table 1 Appendix C for full data on the degree of organization in materials from canisters exposed to all the various environments - including "ground" and "synchronous" controls as indicated in Table 1). The counts confirmed our visual subjective assessment that there was no major difference between the 0 and 1 g control dishes. Fig. 19 shows these data in the form of histograms. Standard deviations will give an idea of the degree of variation that occurred in the clone of cells used under these conditions. Table 3 provides data concerning the number of organized forms encountered in relation to the location of a particular dish in a given canister. The data as analyzed shows that no statistically significant differences existed among, or within, the treatments. Neither was there any major effect attributable to the position of a given dish in the canister.

From prior experience with the clone and especially, the initial data which was summarized in Tables 2 and 3 and Fig. 11, it was appreciated that there was a substantial range of variability within each of the arbitrary categories 1 through 4. Therefore, special care was taken to allocate each of the forms to a specific group. Even so, it was decided that, to better distinguish any potential differences due to treatments, even more categories were needed than the four first established and utilized at the outset.

Table 2. Degree of Organization in Material from Canisters Exposed to Zero g and One g. Numbers refer to organized forms subjectively scored stages "1" to "4". Data represent the average number of individual forms counted per dish in a sample of 5 dishes.

Experimental Treatment	Stage 1	Stage 2	Stage 3	Stage 4	Average Total Number of Organized Forms/Dish
USSR Flight 0 g (KF-1)	1221 ± 336	336 ± 119	152 ± 65	94 ± 37	1803 ± 299
USSR Flight 1 g (KF-2)	1131 ± 263	269 ± 45	173 ± 44	98 ± 26	1671 ± 239

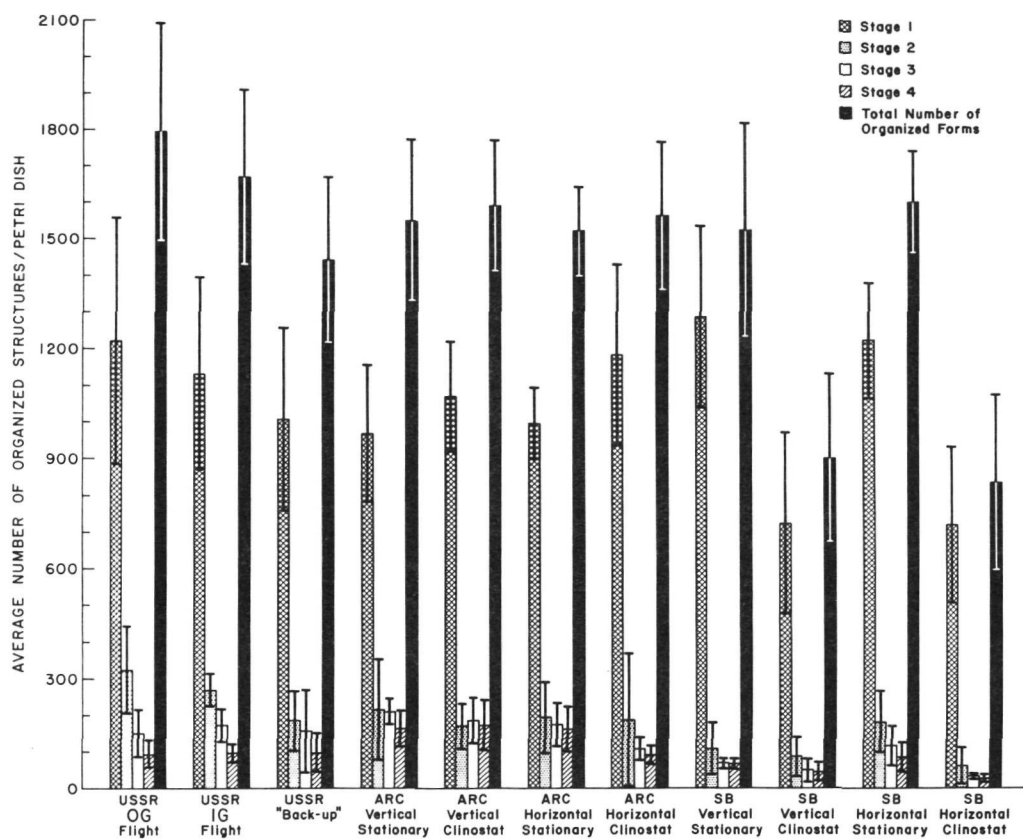


Fig. 19. Degree of organization in material from canisters exposed to various environments (cf. Table 1). For the purpose of this report, attention has been directed only to the first 2 bars of the complete group of histograms. This is appropriate since only these two treatments are strictly comparable. The data represent the average number of individual embryonic forms counted per dish in a sample of 5 dishes. Reference may be made to Figs. 9 a to f for a photograph of the structures in each broad category.

Table 3. Organized Forms in Relation to Dish Location in Canister. Numbers refer to organized forms subjectively scored from stages "1" to "4". Data represent the average number of individual forms counted per dish in a sample of 5 dishes.

Dish Position in Canister		Stage 1	Stage 2	Stage 3	Stage 4	Average Total Number of Organized Forms/Dish
Top	1	1008 $\pm$ 204	281 $\pm$ 127	114 $\pm$ 70	90 $\pm$ 46	1493 $\pm$ 221
	2	1170 $\pm$ 303	177 $\pm$ 121	126 $\pm$ 69	109 $\pm$ 71	1582 $\pm$ 409
	5	915 $\pm$ 334	185 $\pm$ 113	143 $\pm$ 88	100 $\pm$ 49	1343 $\pm$ 390
	6	1199 $\pm$ 170	101 $\pm$ 62	110 $\pm$ 58	79 $\pm$ 43	1489 $\pm$ 259
Bottom	8	989 $\pm$ 273	155 $\pm$ 74	156 $\pm$ 89	120 $\pm$ 79	1420 $\pm$ 425

Several experimental dishes were still available for further manipulations. The agar medium with its organized structures embedded in it was gently released from the plastic petri dishes by means of a spatula and transferred to a large glass petri dish (10 cm) containing hot water. By careful tapping on the agar after it had been allowed to imbibe the hot water for a few (2 or 3) minutes, the embryos could be separated from the agar softened in this way. Embryos which would have been merely categorized as stage 4 in the earlier examinations, were now separated into 10 sub-categories based on their size and shape. Stage 3 embryos were also removed as 3 separate categories; the same for stage 2. Stage 1 embryos were segregated into 2 categories. The rationale for the categories, again, was subjective but it was based on the principal that the more forms on which a direct comparison could be made between embryos that developed in space under 0 g and 1 g conditions, the greater would be the opportunity to detect differences. Figs. 20 to 23 provide a direct visual comparison of the 18 categories of embryonic forms so separated. The left hand side of the figures represent those structures which developed under near-zero gravity conditions; the right hand side represents those which developed under 1 g conditions on the centrifuge in the space capsule. The figures show in vertical sequence the stages of the embryos as they were arbitrarily classified. The first two embryonic forms are heart-shaped; the next three forms are torpedo-shaped; the next three forms are still more advanced and comprise a later torpedo stage; the last ten forms are all more or less well-developed embryos and plantlets, some of which have very long roots. But whatever their special features or stages of development it will be noted that the forms which developed under near-zero g or at 1 g conditions on the centrifuge in space are very similar. Moreover, the numbers of embryos developed at each stage at 0 g or 1 g were not significantly different.

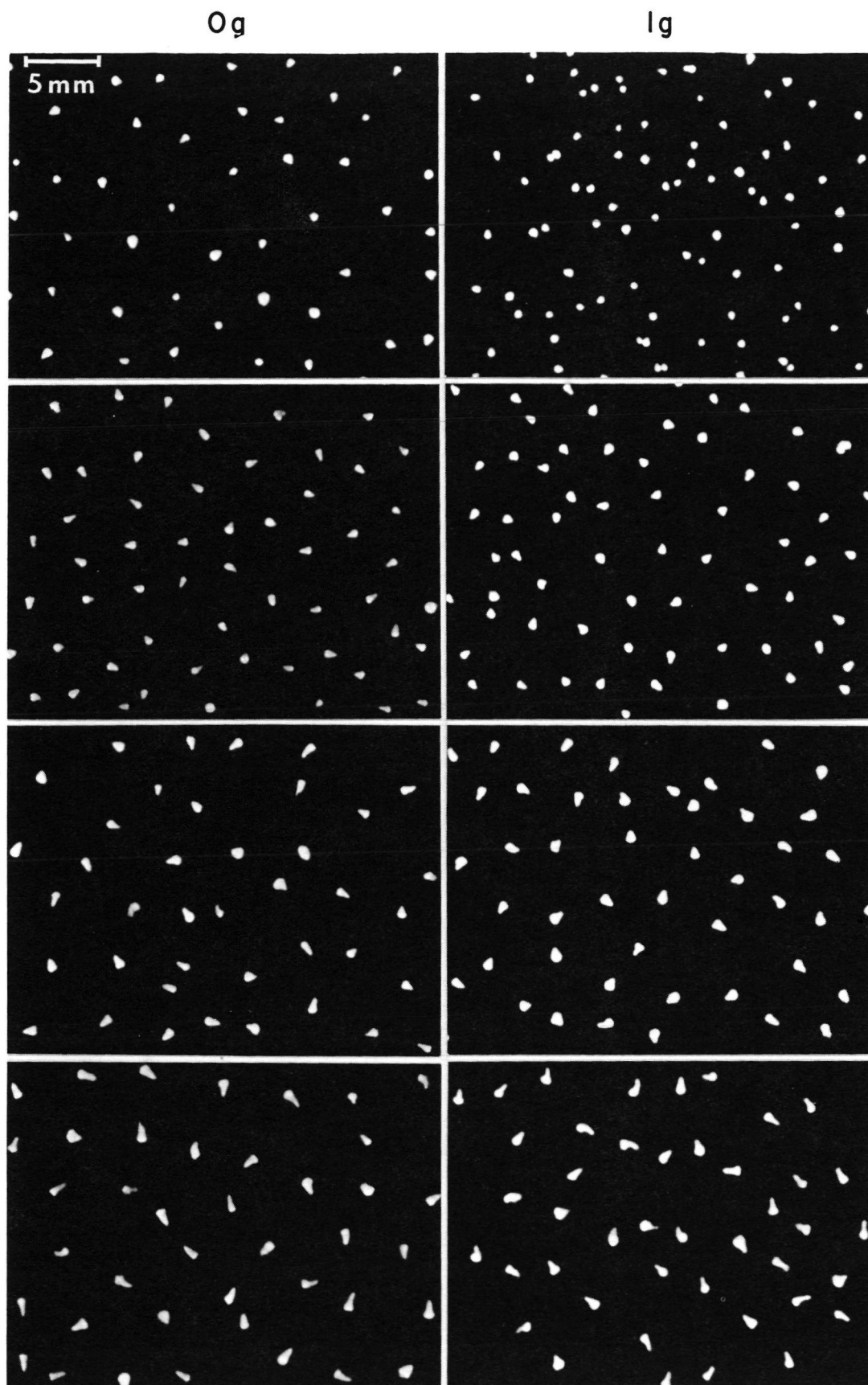


Fig. 20. Embryonic forms from dishes exposed to near-zero g (left column) and 1 g (right column) conditions in space separated into arbitrary categories and arranged to facilitate ready comparison.

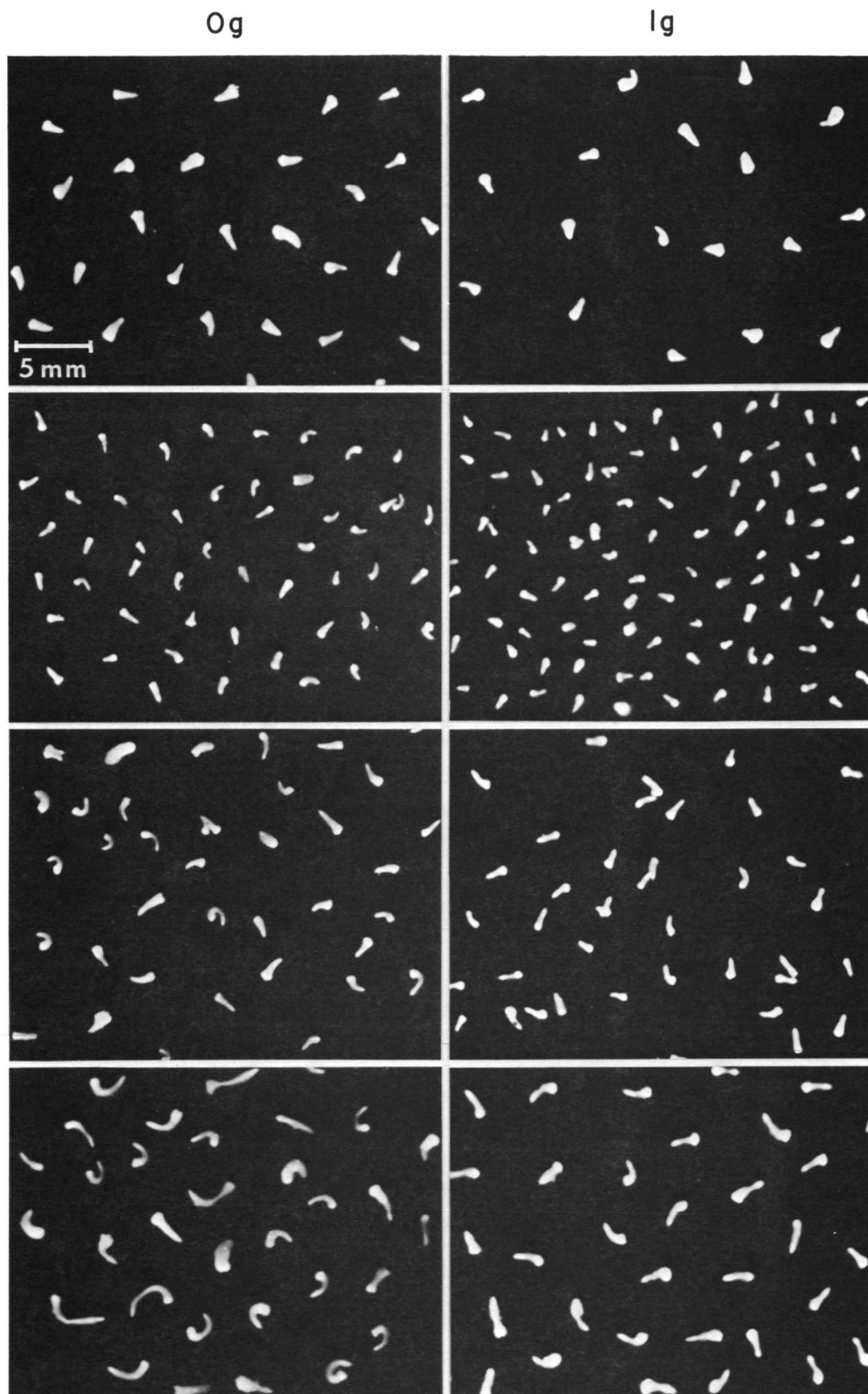


Fig. 21. Embryonic forms from dishes exposed to near-zero g (left column) and 1 g (right column) conditions in space separated into arbitrary categories and arranged to facilitate ready comparison.

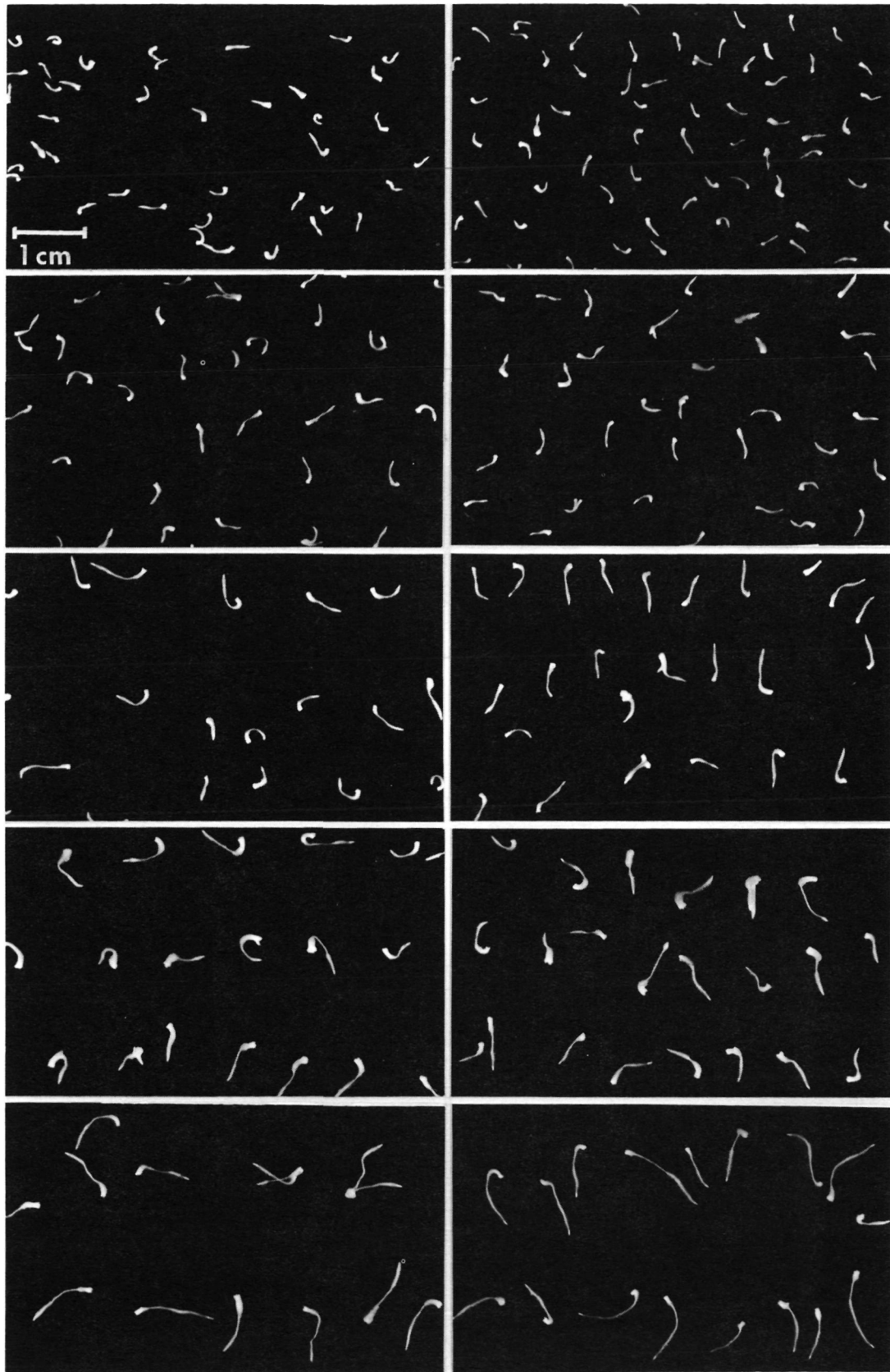


Fig. 22. Embryonic forms from dishes exposed to near-zero g (left column) and 1 g (right column) conditions in space separated into arbitrary categories and arranged to facilitate ready comparison.

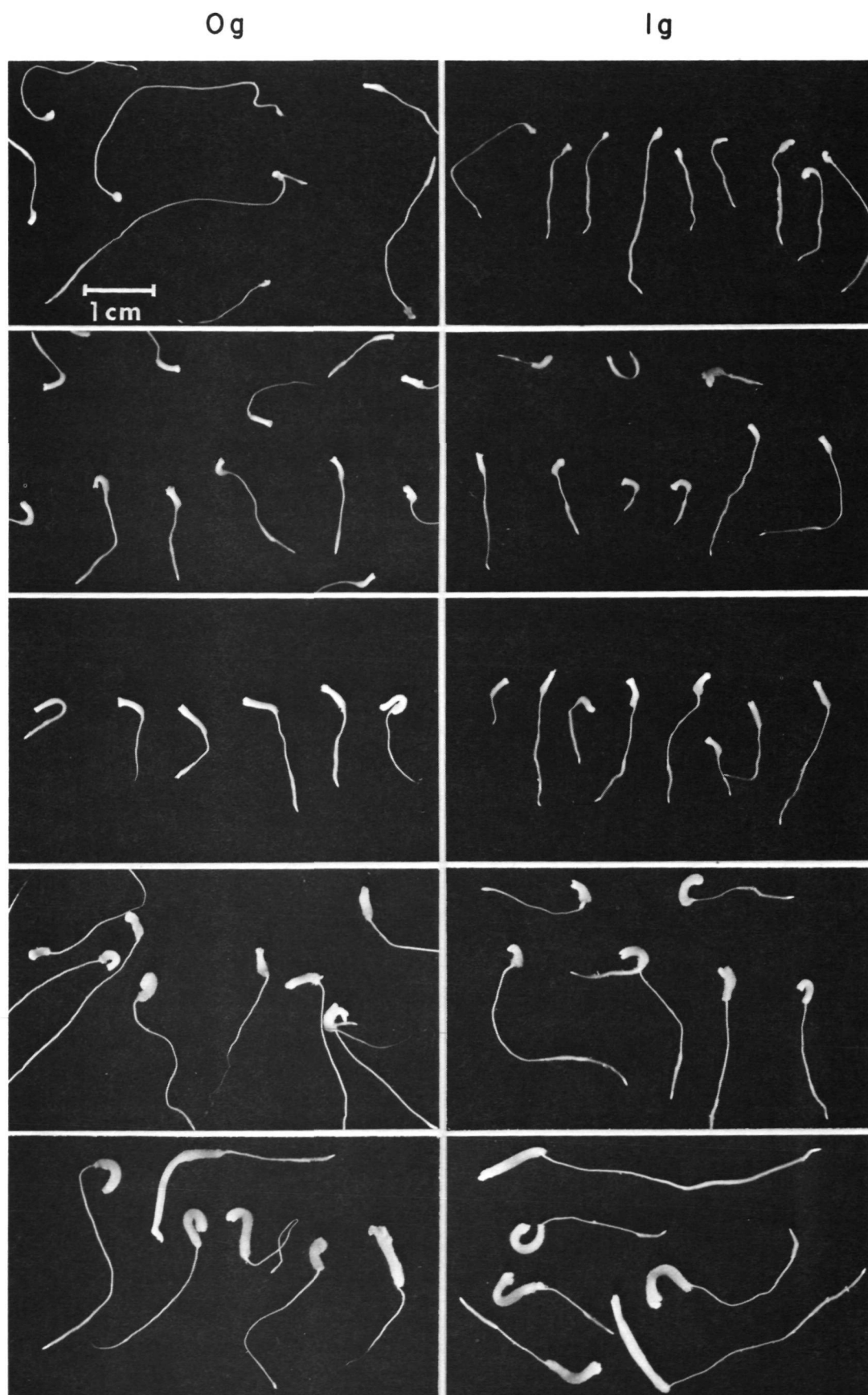


Fig. 23. Embryonic forms from dishes exposed to near-zero g (left column) and 1 g (right column) conditions in space separated into arbitrary categories and arranged to facilitate ready comparison.

As an example, special attention was paid to the category 4 of more advanced embryos to detect whether this may have included embryos which emphasized to different degrees a very great elongation of true roots or an enlargement of the hypocotyl. It is clear from the photographs that all those in any given category, are, however, similar and have features in common. Nevertheless no differences could be detected, even upon careful direct comparison, between those forms which emerged during growth at near-zero g and the 1 g centrifuge.

Still other petri dishes were used as sources of material to test the subsequent viability and growth responses of the crops of embryos that developed in space. Embryonic forms categorized as 1 to 4 were allowed to continue their growth on fresh media, first in small 50 mm plastic petri dishes, later in tubes and still later in pots under greenhouse conditions. After a week or so of growth in darkness, the embryos, whatever their initial stage of development at the time of their removal from the experimental dishes, and subsequent "planting" on fresh media quickly attained a comparable size. In fact, it was not easily possible to distinguish those plantlets which were derived from stage 1 or stage 4 embryos, except by checking the laboratory records. Figs. 24 and 25 provide a photographic record of representative stages of growth from aseptically cultured embryos to plants in pots in the greenhouse. The Figures show:

- (a) 4 dishes, each containing embryos which arose during flight, were selected at stages 1, 2, 3, and 4 respectively and later grown approximately one month on fresh media. Note that the original differences between the embryos have been minimized as they grew.

- (b) A large culture tube containing plantlets shortly after their transfer from one of the dishes shown in (a).
- (c) A large culture tube containing plantlets after 40 days growth in the tube.
- (d) and (e) Show subsequent growth of the carrot plants in pots after 1 and 2 months respectively.

No attempt has been made here to provide a "case history" for each of the embryos brought to maturity but a photographic record exists in the laboratory files. Again the results were clear. There was no detectable difference between the subsequent ability of any of the stages of embryos which had developed under space conditions at zero or one g to continue their course of growth and development. The plantlets are within normal range of variability for wild carrot. Indeed the plants look identical for they are the products of a now well-established cloning procedure(cf. Steward and Mapes, 1963). Dozens of plantlets were grown in the laboratory and greenhouse in order to have materials for observations on potential variations in gross morphology. McClintock (1968) has drawn attention to the variability of the distribution of anthocyanin pigment within flowers of the umbel of "Queen Anne's lace". Purple pigment is often present in petals in flowers in the central region but the extent of its occurrence varies widely in umbels of different plants. When our plants flower, special attention will, therefore, be paid to the distribution of pigmented florets. Hitherto, the chromosome numbers of roots of plants grown from cultured carrot cells have all been of the normal diploid, $2n = 18$ (Mitra et al., 1960; Mulligan, 1961; Whitaker, 1949; Bell and Constance, 1960). Pending nuclear cytology, which has not yet been done, some leaves have been cleared

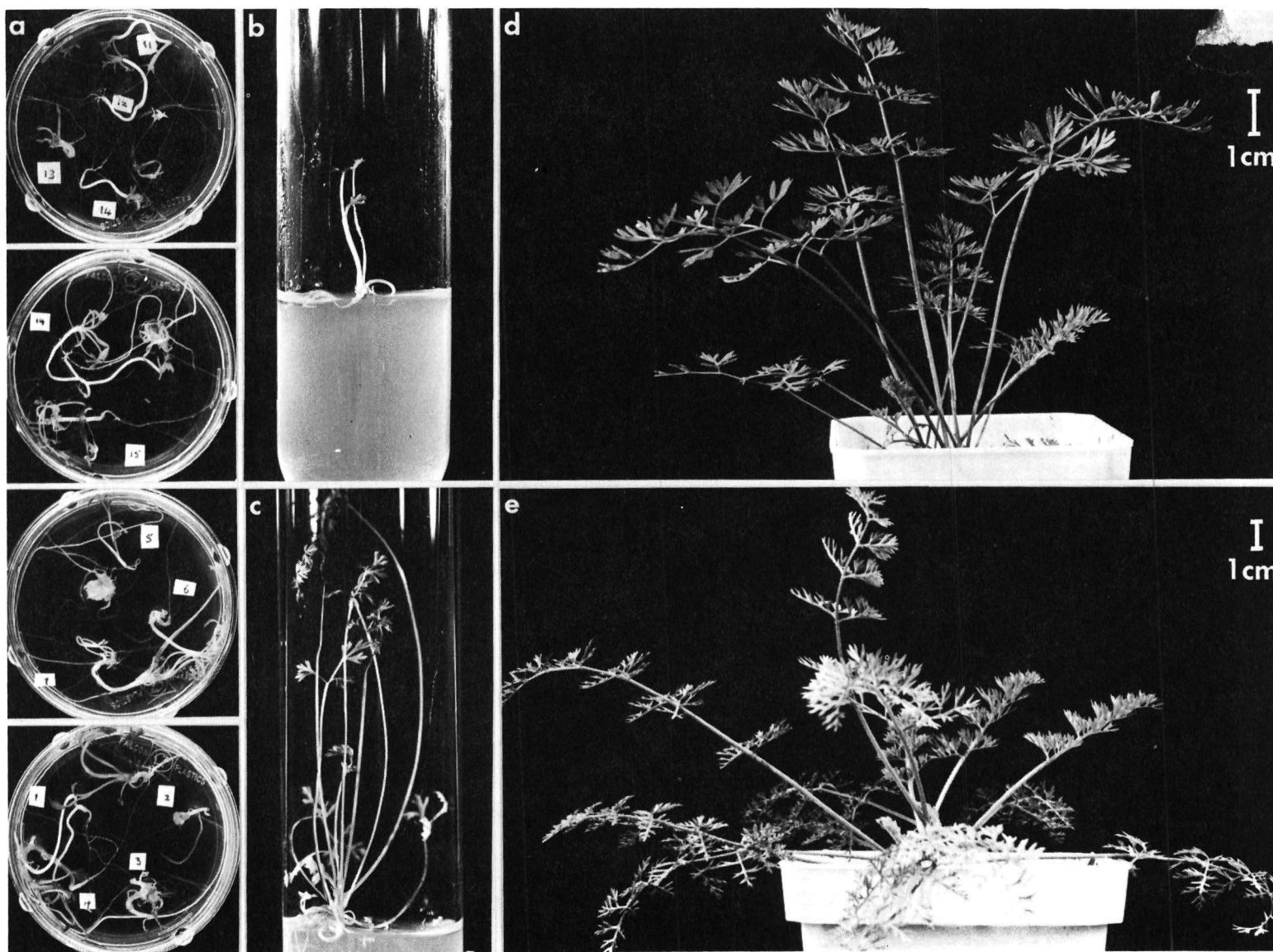


Fig. 24. The development of carrot plants from embryos which developed in space under conditions of near-zero g. See text for details.

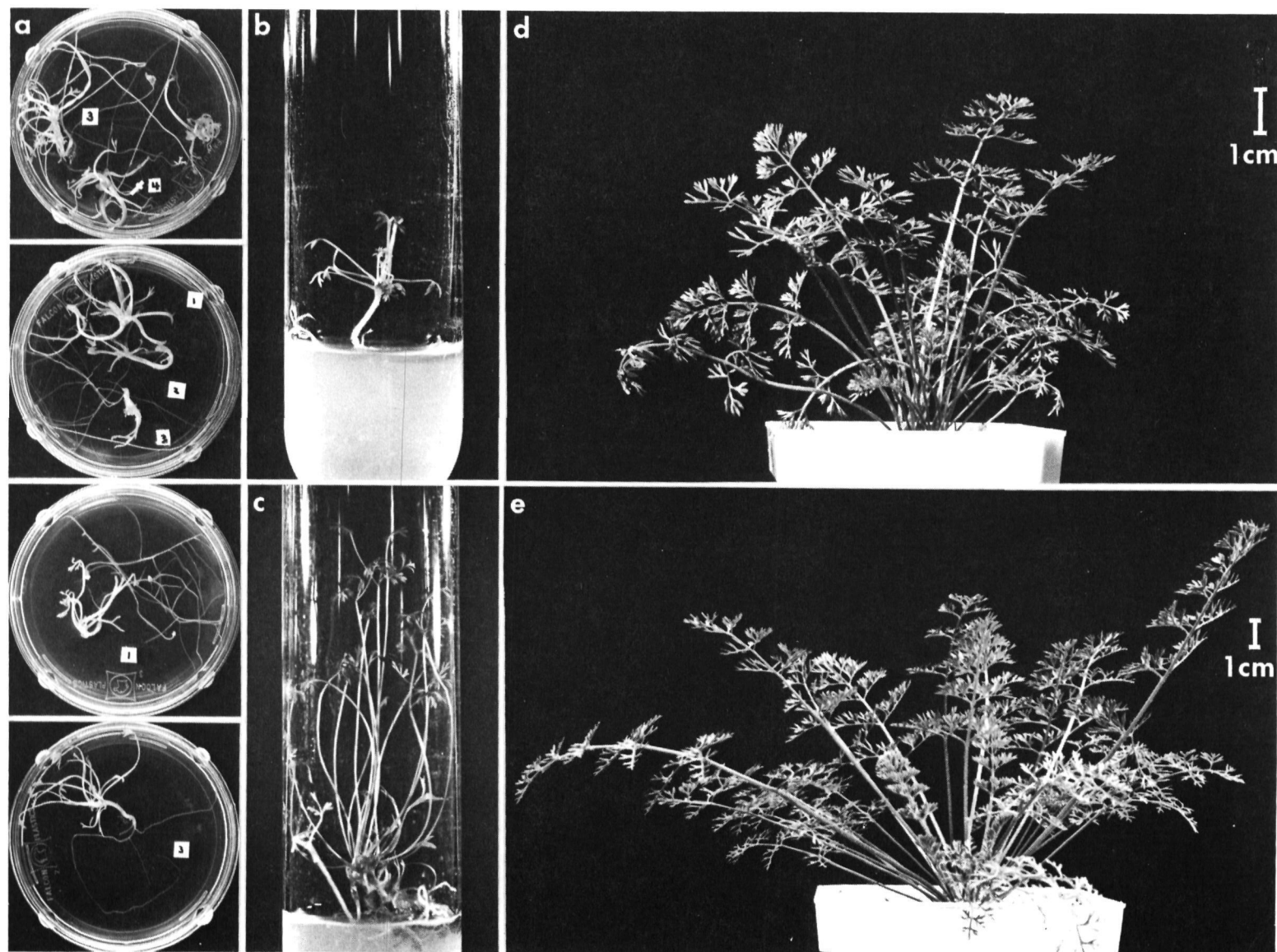


Fig. 25. The development of carrot plants from embryos which developed in space under 1 g conditions on a centrifuge. See text for details.

and stomates examined for size differences since it is well known that changes in ploidy level can often be distinguished in higher plants by examining size and distribution of stomates (cf. also Dudits et al., 1976). To date, and as expected, no such differences have been detected between leaflets of "zero g" and "1 g" carrot plants.

DISCUSSION

The Cosmos 782 experiment addressed the fundamental question whether a complex sexually-reproduced higher plant can achieve the full complexity from single cells (whether zygotes or somatic cells) in the weightless conditions of space. As man moves increasingly into space, the answer to such a question will have major implications for an ultimate "space agriculture" using crop plants. The question also has general significance in terms of morphogenesis in any organism in space.

But it is not yet practical to utilize even a small seed plant such as Arabidopsis thaliana that normally completes its life cycle from seed to seed in a period of about 21 days to provide an answer to this question (cf. Merkys, et al., 1975). Moreover, by the time seeds have formed, highly organized structures with well-defined growing regions of shoot and root apices have already developed. Therefore, to investigate the most important early stages of development, this ought to be done before the organized growing regions have arisen. It is now often more feasible to grow entire plantlets from vegetative cells than to do so using isolated, fertilized, eggs or even zygotes in situ (i.e., in embryo sacs in ovules). The large number of somatic embryos which can be grown from cultured carrot cells in a small space are

especially well adapted for the study of the effects of the space environment, especially near-zero gravity, on development. In this experiment, we look upon the behavior of isolated totipotent cells of carrot as equivalent to that of free fertilized eggs as they might develop and grow when isolated from the asymmetries of their normal situation in situ.

The Cosmos 782 Biological Satellite afforded a unique opportunity to perform an important direct experiment at near zero gravity in space. This was not only because of the relatively long period of flight (19.5 days), but because of the presence of a centrifuge aboard the spacecraft that would expose cells, which would serve as rigorous controls, to a gravitational force equivalent to that on earth (1 g).

The carrot cell culture material actually recovered from Cosmos 782 was intact and in good condition. This was no mean achievement in view of the major logistical problems posed by performance of the experiment from the U.S.S.R. despite the actual preparation of the cells at Stony Brook, N.Y. and their transmittal to the Soviet Union via California. There has been no reason to date to suppose that the behavior of the cells has been limited in any way by untoward or accidental circumstances such as radiation during the period of the flight. (This is relevant because it was shown that gross structural changes could occur in tobacco mosaic virus. Gaps in the structural coat or splitting of the viral rod into smaller units were the attributed result of solar ultraviolet radiation (cf. Orlob, 1969).)

The behavior of the clone of wild carrot cells finally used for this experiment may not, at first glance, appear as aesthetically satisfying as that of any number of our other clones that were more uniform in their developmental behavior

(cf. Fig. 26). But the clone actually used provided an opportunity to evaluate many embryos at many stages of development. This would have only been possible if any of several distinct clones which were more uniform in their response were flown for longer periods of time. Therefore, the cells actually selected for use were, in fact, well suited to the experimental test as it was carried out.

Based on our laboratory findings with the clone of wild carrot cells as used in this experiment, it is suggestive that most of the embryonic development from cells occurred during the first 14 days of flight (cf. Figs. 16 to 18). It is noteworthy here, however, that the pace of development in normal zygotic embryogenesis in the wild carrot is equalled or even surpassed by the asexual somatic embryo system (unpublished observations). It is, however, a potentially significant point that this early development of embryo-like forms from cells (that occurs in 14 days, or so, in complete darkness) concerns the general form of the embryo whereas concomitant tissue differentiation (especially that of protoxylem strands intimately associated with the development of leaf primordia) may, under these conditions be arrested. Thus it may be one important problem, and achievement, to establish whether or not embryonic form (embryonic induction) occurs equally at zero g as at one g in the dark whereas it may be another problem to establish how far later light dependent development of the preformed shoot is, or is not, sensitive to 0 g conditions.

The crucial observation made is that no significant differences occurred between the development of carrot cells in the dark between 1 g and 0 g conditions in flight. The possibility that no growth of any kind occurred during flight (i.e. that it had already occurred prior to lift-off in the

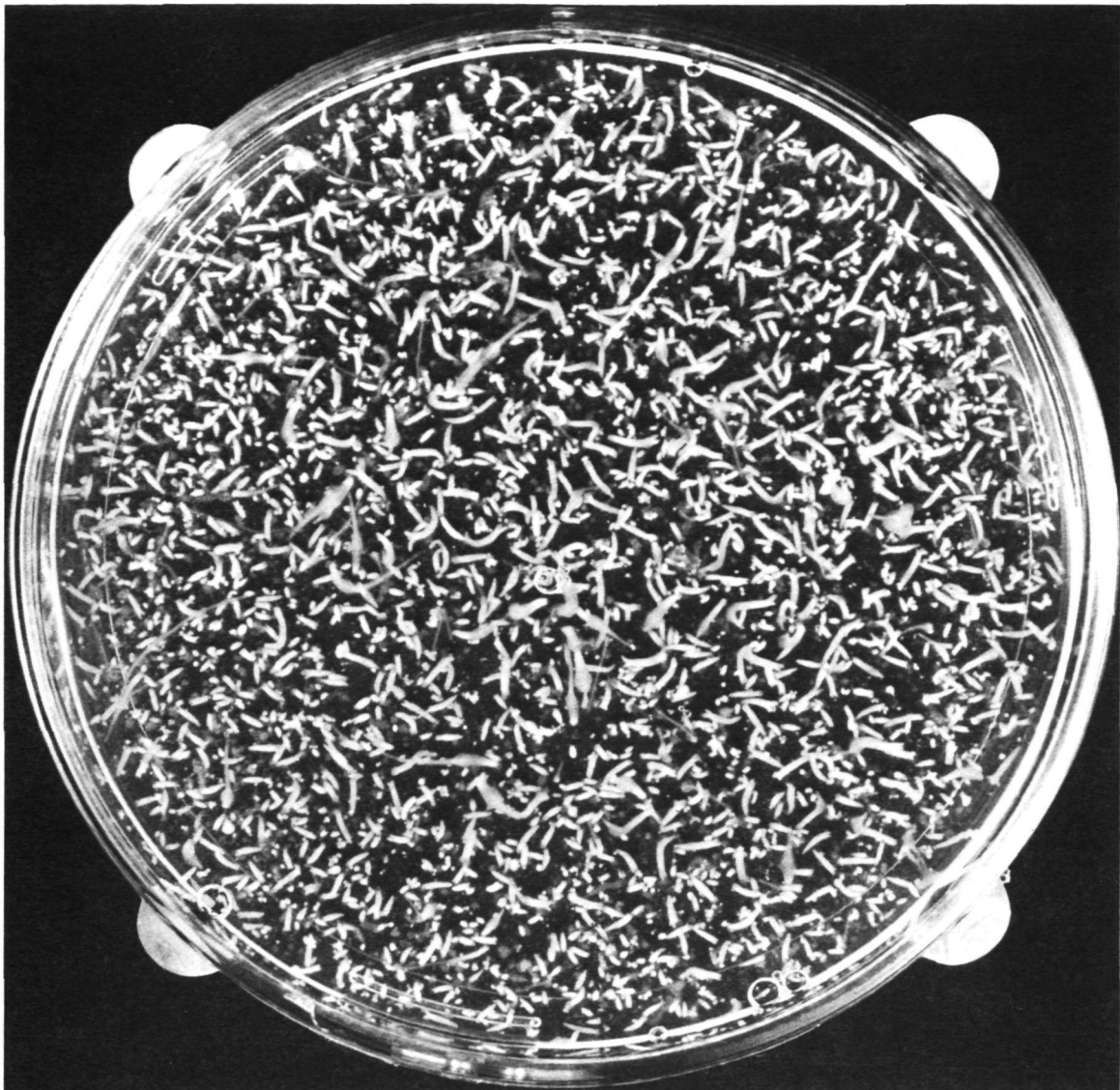


Fig. 26. Shows a large number of young somatic embryos after they had developed in darkness from free cells or small units within a shallow layer of agar medium, in plastic petri dish 50 mm in diameter during 19.5 days. This clone of cells derived from a stamen filament of wild carrot. Note the large crop of relatively uniform embryos.

Biotransporters) does not exist. The only remaining dilemma, however, seems to be as follows: could cells subjected to an asymmetric 1 g stimulus (as in the Biotransporters at $4^{\circ}\text{C} \pm 2$ for 6 days and 14 1/2 hours prior to lift-off at "ambient" room temperature) receive an implanted 1 g stimulus or "message" (i.e. during this protracted "presentation time") which could allow them when they did develop subsequently to do so independently of any gravitational stimulus? Young (1976) has raised similar important questions with special reference to experiments performed in space using animal embryos.

Finally, the question of the development of shoots in space on embryos grown from cells arises. It is clear that cells can give rise to embryos and small plantlets with a copious development of roots in about 19.5 days in darkness and at near-zero g. Such embryos and plantlets readily initiated shoot growing points which, when restored to 1 g and in the light, developed rapidly into normal green shoots. But the test of the competency of the near-zero g environment to support normal growth and development is not really complete until it is extended into the further growth of embryonic shoots in the light. The test would, again, require longer periods of flight and the use of controlled light at minimal predetermined levels for morphogenesis. In any event, however, if any differences could be disclosed, they would concern a problem of late development from already-organized heart-shaped structures, and not their induction de novo. Our Cosmos-782 experiment, perforce, could not concern the later development of shoots which characteristically requires light in a fully autotrophic system.

The very fact that higher plants, which have evolved under conditions of 1 g, now utilize interactions with

different environmental parameters that control development and metabolism here on earth renders it presumptions to conclude that the problems of biology in outer space are minimal. There simply have not been enough carefully controlled experiments of long enough duration to justify this attitude. In the case of this carrot cell culture experiment with its limitations as pointed out in this discussion, it would seem that a gravitational field is not necessary for totipotent cells to undergo morphogenesis. But the limitation of the experiment as performed is very real and an important task still remains to ascertain whether this will be so under conditions of longer flight and development in the light. Even in the dark and by passage through more than one, or several "life cycles" in space, one could test whether there are any residual effects attributable to a sort of "memory" of the gravitational stimulus to which cells of embryo plantlets were initially exposed as cells on earth.

ACKNOWLEDGMENTS

This experiment could only take advantage of the Cosmos-782 opportunity because of long prior experimentation to adapt the experimental system to the anticipated conditions of space flight. This was made possible by a series of NASA contracts initially to one of us (F.C.S.) at Cornell University, Ithaca, N.Y. and latterly at Stony Brook to both of us for our collaboration (Contract NAS2-8986), at the State University of New York at Stony Brook, N.Y. All the work prior to the Cosmos-782 flight at Stony Brook involved the indispensable help and loyal support of Mr. F. Ronald Dutcher and Miss Elizabeth T. Yim who, having developed the necessary skills, gave us the needed technical assistance. On all matters concerning engineering design, the pre-testing of the experimental system and package as installed in the spacecraft we were in close collaboration and invariably helpful relationships with NASA officials at Moffett Field, Ames Research Center, California, especially Dr. John W. Tremor and Dr. Richard C. Simmonds. Lastly, after the Cosmos-782 flight plan was initiated, we enjoyed the full cooperation of the appropriate officers of the U.S.S.R. especially in the Institute of Biomedical Problems and the K.A. Timiriazev Institute of Plant Physiology as directed by Dr. Olaf Gazenko and by Dr. A.L. Kursanov respectively. In the latter Institute, Professor Dr. R. G. Butenko made available to us with Dr. N. Dimitriaeva, when necessary, the resources of her laboratory and also functioned as our scientifically informed contact in the U.S.S.R.

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Appendix A. General protocol for preparation of carrot cell material for microscopy as utilized by Dr. Myron C. Ledbetter, Biology Department, Brookhaven National Laboratory, Upton, L.I., New York 11973.

Detailed sequence of procedures

(see following sheets for exact method of preparation of buffers)

- 1) Flood the plastic petri dish of agar containing material with 1% buffered glutaraldehyde (in .05 M Sorensen Phosphate Buffer) for 1 to 2 hours at room temp. (or longer, overnight is acceptable).
- 2) Remove agar containing cellular or organized materials (1/2 dish or 1/4 dish) in tiny pieces and place into centrifuge tube. Add 3% buffered glutaraldehyde (in .05 M Sorensen Buffer) for 1 to 2 hours.
- 3) Centrifuge for 10 min. (about 300 rpm). Remove glutaraldehyde. Replace with fresh 3% buffered glutaraldehyde. Heat for 10 min. in 45°C water bath. (At this point, the materials may then be stored undrained in 4-5° C refrigerator indefinitely).
- 4) At least three changes (it is best to use 5 or 6) of .05 M phosphate buffer wash should be made. 20 min. each, then centrifuge for 10 min., suck off the wash with a pipette, add fresh wash, etc..
- 5) Treat with 2% osmium tetroxide in .05 M Sorensen buffer for 2 hrs. or as long as overnight at room temperature.
- 6) Dehydration. Chill material. Keep acetone and test tubes in ice bath. Do two changes of 10% acetone (30 min. each) then 30 min. of 30%, 50%, 70%, 90%, 100% acetone. Do several changes of 100% using new bottle of acetone for final one to be sure there is no water. May store in refrigerator at 70%, 90% or 100%.

- 7) Embed in epoxy plastic according to Luft (1961, revised 1968)

	<u>Luft A</u>	<u>Luft B</u>
Epon Resin 812	66 g	100 g
DDSA	100 g	--
NMA	--	84 g

May store A and B separately for 6 months in refrigerator.

To use, mix A and B together at room temperature.

A 7 g

B 3 g

DMP 30 .15 ml

Bring to room temp. in 100% acetone

30% plastic 70% acetone $\geq$ 30 min.

50% plastic 50% acetone $\geq$ 30 min.

70% plastic 30% acetone $\geq$ 30 min.

100% plastic $\geq$ 30 min.

100% several more changes

Rotate all the above for best results

- 8) Pour into capsules, set in oven at $60^{\circ}\text{C} \approx 48$ hrs.

Summary Sheet Describing Fixation and Embedding Methods Utilized

Buffers: Sorensen phosphate at pH 6.8
(see attached sheet for details of preparation)

Code Designation of "Fixative"	Solution	Time	Temperature
"SPG I"	1% buffered glutaraldehyde in .05 Molar Sorensen Phosphate Buffer	1 to 2 hours	Room temperature (15 to 25°C)
"SPG II"	3% buffered glutaraldehyde in .05 Molar Sorensen Phosphate Buffer	1 to 2 hours	Room temperature (15 to 25°C)
"SPG II"	3% buffered glutaraldehyde in .05 Molar Sorensen Phosphate Buffer	10 minutes	Heat for 10 minutes in a water bath at 45°C
Allow to cool to room temperature. Note: This may also be stored in a refrigerator at 4-5°C. Theoretically the material thus treated can be stored indefinitely. In order to completely process the plant cell materials, the following needs to be performed:			
	3 changes of .05 Molar Phosphate buffer "wash"	1 hour total	20 minutes each at room temperature
	2% osmium tetroxide (OsO ₄) in .05 Molar Sorensen Phosphate buffer	2 hours or as long as "overnight"	Room temperature
	Dehydrate in Acetone 10, 30, 50, 70, 90, 100%	30 minutes for each dehydration	Ice bath utilized
(May be stored in refrigerator or deep freeze at stage of either 70, 90 or 100% Acetone)			
	Embed in Epoxy plastic according to Luft (1961)		

Exact Details of Preparation of Sorensen Phosphate
Buffer "Stock" Solutions to be Utilized in Preparation
of "SPG I" and "SPG II" Fixatives

Sodium Phosphate Monobasic ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)	28.2 Gm
Sodium Phosphate Dibasic (Na_2HPO_4)	27.8 Gm Anhydrous or 52.5 Gm if $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ used
Distilled water to make	1000 milliliters

Note: This makes 0.4 Molar phosphate buffer

Exact Details of Preparation of 1% Calcium Chloride (Anhydrous)
Use in Preparation of "SPG I" and "SPG II" Fixatives

Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)	1.325 Gm.
Distilled water to make	100 milliliters

Precise Details of Final Preparation of Sorensen Phosphate Fixatives Designated "SPG I"
and "SPG II"

In order to prepare any of the following, utilize:

	<u>Molarity</u> <u>Desired</u>	<u>Glutaraldehyde</u>	<u>Sorensen Phosphate</u> <u>Stock Solution</u>	<u>1% Calcium</u> <u>Chloride (for</u> <u>.001 Molar)</u>	<u>Glutaraldehyde</u> <u>25% Practical</u> <u>Grade</u>	<u>Final</u> <u>Volume</u>
"SPG I"	0.05	1%	5ml.	0.4ml (12 drops)	1.6ml.	Distilled water to 40 milliliters
"SPG II"	0.05	3%	5ml.	0.4ml	4.8ml	Distilled water to 40 milliliters

APPENDIX B. Detailed time Schedule of Preparation, Transmittal, Flight
and post-flight Operations, with Special Reference to Temperature

I. Preparation of Biological Materials and
Introduction of Petri Dishes into Canisters

1975

Nov. 19 The biological materials were all prepared
at Stony Brook. Canisters KF-1 through 10
were placed in the biotransporter at
approximately 20:00 EST.

Temperature

approx. 4°C

Canisters KF-11 through 14 were placed in
a 4°C refrigerator.

4°C

II. Transmittal of Canisters KF-1 through 10 to Ames
Research Center (ARC)

Nov. 20 The biotransporter was shipped to ARC (with
Mr. Darren Waters).
After arrival, the units were placed in
a 4°C incubator while the coolant was changed.

Approx. 4°C

4°C

III. Transmittal of Canisters, KF-1 through 6 to Moscow, USSR
(with Dr. John Tremor)

Nov. 21 Canisters KF-1 through 6 were equally dispersed
between two biotransporters: KC46 and KC47

KC46 (KF-1, 3, 5) KC47 (KF-2, 4, 6)

08:15 (11:15 EST) San Francisco Baggage Claim Area
08:30 (11:30 EST) Claim Area
08:50 (11:50 EST) San Francisco Ramp
09:10 (12:10 EST) San Francisco Ramp
09:45 (12:45 EST) Take-off

5.2°C 6.3°C
5.2°C 6.0°C
5.2°C 5.9°C
4.8°C 5.5°C
- -

Nov. 22 06:55 (01:55 EST) London; the temperature was read
by an agent on the baggage ramp
10:00 (05:00 EST) Baggage door of plane
22:05 (14:05 EST) Moscow ramp, outside baggage area

4.1°C 4.2°C
3.9°C 4.0°C
4.4°C 4.4°C

					<u>KC46 (KF-1,3,5)</u>	<u>KC47 (KF-2,4,6)</u>
Nov. 23	12:00	(04:00 EST)	Institute for BioMedical Problems		4.6°C	4.6°C
	14:00	(06:00 EST)	" " " "		4.5°C	4.7°C
All canisters were moved into biotransporter KC47.					--	(KF-1 through 6)
	16:50	(08:50 EST)	Institute for BioMedical Problems		--	4.5°C
	17:25	(09:25 EST)	" " " "		--	4.6°C
	17:55	(09:55 EST)	" " " "		--	4.6°C
Canisters KF-1 through 4 were removed into biotransporter KC46; Canisters KF-5 and 6 remained in biotransporter KC47.						
	18:50	(10:50 EST)	Institute for BioMedical Problems		3.9°C (KF1-4)	4.6°C (KF5&6)
	19:15	(11:15 EST)	" " " "		5.1°C	5.9°C
	19:30	(11:30 EST)	" " " "		4.6°C	5.3°C
Nov. 24	09:15	(01:15 EST)	" " " "		4.5°C	4.5°C
	09:30	(01:30 EST)	" " " "		4.6°C	4.7°C
	09:40	(01:40 EST)	Canisters KF-5 and 6 were moved from biotransporter KC47 into a 4°C refrigerator			
	10:10	(02:10 EST)	Institute for BioMedical Problems		6.3°C	
	10:30	(02:30 EST)	" " " "		6.0°C	
	10:35	(02:35 EST)	" " " "		5.9°C	

Biotransporter KC46, containing the flight and back-up canisters KF-1 through 4, left the Institute for the launch site.

IV. Space Flight, recovery of spacecraft, and return of flight canisters KF-1 and 2 to the United States.

Nov. 25. 09:10 - 09:30 (01:10 - 01:30 EST) The canisters were removed from the biotransporter KC46 and were loaded into the flight containers. From that time until launch, the loaded canisters were kept at ambient room temperature. Back-up canisters KF-3 and 4 were kept in uncontrolled temperature conditions. All manipulations performed outside Moscow were done by Soviet technicians.

20:00 (12:00 EST) Launch. The centrifuge was started as soon as orbit was achieved, only "minutes after launch."

Ambient Room
Temperature

Daily maximum-minimum temperature within the spacecraft

<u>Day of Flight</u>	<u>Temp. (in vicinity of "biology")°C</u>		
	<u>Minimum</u>	<u>Maximum</u>	<u>Average</u>
1 (Nov. 26)	x	x	x
2 (Nov. 27)	x	x	x
3 (Nov. 28)	x	x	x
4 (Nov. 29)	x	x	x
5 (Nov. 30)	x	x	x
6 (Dec. 1)	x	x	x
7 (Dec. 2)	x	x	x
8 (Dec. 3)	19.5	21.2	20.4
9 (Dec. 4)	19.2	22.0	20.6
10 (Dec. 5)	19.5	20.0	19.8
11 (Dec. 6)	19.5	21.1	20.3
12 (Dec. 7)	20.0	22.0	21.0
13 (Dec. 8)	19.3	21.3	20.3
14 (Dec. 9)	18.9	21.5	20.2
15 (Dec. 10)	18.6	21.0	19.8
16 (Dec. 11)	19.3	20.7	20.0
17 (Dec. 12)	19.6	22.7	21.2
18 (Dec. 13)	21.7	22.5	21.1
19 (Dec. 14)	22.0	23.2	22.6
20 (Dec. 15)	*	*	*

* Data is not available due to engineering/telemetry problem.

Temperature

Dec. 15 02:42 (18:42 EST) The centrifuge was stopped (about
Dec. 14) 300 minutes before re-entry).
08:58 (00:58 EST) The landing (R) occurred.
R+23 minutes. The first recovery team reached the spacecraft.
R+75 minutes. The spacecraft was covered with the Mobile
Laboratory Tent.
R+210 minutes. The Mobile Laboratory was set up and the
spacecraft unloading began.
R+340 minutes. Canisters KF-1 and 2 were unloaded.
R+390 minutes. The processing was completed.
15:30 - 15:45 (07:30 - 07:45 EST) The canisters were placed
in 4°C transporter.

approx. 4°C

Dec. 16 R+27 hours. The flight samples left the landing site airfield.
R+33 1/2 hours. The flight samples arrived in Moscow.

Dec. 17 about 11:00 (03:00 EST) The canisters were delivered to the
U.S. and Soviet co-investigators at the K.A.
Timiriazev Institute of Plant Physiology in
Moscow

Dec. 19 17:00 (09:00 EST)

7°C-The temperature
was reportedly stab-
ilized at 4.7°C by
the time Soviet
technicians left the
facility.

Dec. 20 16:00 (08:00 EST)

4.4°C

Dec. 21 10:00 (02:00 EST)

4.4°C

11:00 (03:00 EST) New heat sinks were inserted into the
biotransporter.

11:15 (03:40 EST)

7°C

11:40 (03:40 EST)

5°C

12:00 (04:00 EST)

3.7°C

14:30 (06:30 EST)

3.5°C

on plane
The heat circuit was operative and working
when loaded onto the plane in Moscow (in
the passenger compartment.)

Temperature

Dec. 21 (continued) 15:30 (07:30 EST)
16:30 (08:30 EST)

3.5°C
3.5°C

At Copenhagen, the biotransporter was transferred to the forward baggage compartment and the heat circuit was turned off.

24:00 EST (at JFK Airport in New York)

2°C

Dec. 22 00:45 EST Canisters KF-1,2, and 3 were removed from the biotransporter and were placed in a Stony Brook "transporter" (a Coca-cola cooler) which was lined with heat sinks. This "transporter" was kept at ambient room temp.

5.1°C

approx. 4°C

10:30 EST KF-1,2, and 3 were removed from Stony Brook transporter and were placed in 4°C refrigerator. Observations were begun. Individual dishes were removed to room temperature occasionally for observation and were immediately returned to the 4°C refrigerator.

V. U.S.S.R. Synchronous Control Flight and return of U.S.S.R. synchronous control canisters KF-5 and 6 to the United States

Nov. 24 (continued from III) Canisters KF-5 and 6 were in the 4°C refrigerator at the Institute for BioMedical Problems

4°C

Dec. 1 10:00 (02:00 EST) The canisters were removed from the 4°C refrigerator at the Institute and were placed in a 4°C biotransporter.

approx. 4°C

12:00 (04:00 EST) They were removed from the biotransporter.

12:40 (04:40 EST) They were loaded into the "flight" containers.

13:00-15:00 (05:00-07:00 EST) The canisters were subjected to simulated launch stresses.

17:00 (09:00 EST) They were loaded into the synchronous control hardware.

17:40 (09:40 EST) The synchronous control "spacecraft" was sealed.

20:06 (12:06 EST) The synchronous control centrifuge was started.

Daily maximum-minimum temperatures within U.S.S.R. Synchronous Control
"Spacecraft"

<u>Day of Flight</u>	<u>Temperature (in vicinity of "biology")°C</u>		
	<u>Minimum</u>	<u>Maximum</u>	<u>Average</u>
1 (Dec. 2)	21.0	21.0	21.0
2 (Dec. 3)	21.0	21.0	21.0
3 (Dec. 4)	19.7	21.5	20.6
4 (Dec. 5)	19.8	21.8	20.8
5 (Dec. 6)	19.2	21.0	20.1
6 (Dec. 7)	20.7	21.5	21.1
7 (Dec. 8)	19.8	20.8	20.3
8 (Dec. 9)	19.8	20.7	20.2
9 (Dec. 10)	20.3	21.3	20.8
10 (Dec. 11)	20.6	23.1	21.8
11 (Dec. 12)	20.0	21.0	20.5
12 (Dec. 13)	20.2	22.0	21.1
13 (Dec. 14)	20.9	21.3	21.1
14 (Dec. 15)	20.4	21.2	20.8
15 (Dec. 16)	20.4	20.6	20.5
16 (Dec. 17)	20.5	22.0	21.2
17 (Dec. 18)	21.5	21.9	21.7
18 (Dec. 19)	21.9	23.3	22.6
19 (Dec. 20)	22.8	23.8	23.3
20 (Dec. 21)	21.6	23.1	22.4

Dec. 21 02:42 (18:42 EST Dec. 20) The synchronous control "spacecraft" was stopped.
 10:00-12:00 (02:00-04:00 EST) The synchronous control "spacecraft" was unloaded.
 11:00-13:00 (03:00-05:00 EST) The canisters were subjected to the simulated stresses.
 13:30-14:15 (05:30-06:15 EST) The KF-5 and 6 canisters were unloaded.
 14:20-14:30 (06:20-06:30 EST) The canisters were processed.
 17:00 (09:00 EST) The canisters were delivered to the U.S. and Soviet coinvestigators at the K.A. Timiriazev Institute of Plant Physiology.

Dec. 23 A.D. Krikorian returned to Stony Brook with canisters KF-5 and 6 at ambient room temperatures.

VI. ARC synchronous control flight and return of ARC synchronous control canisters KF-7 through 10 to Stony Brook.

Nov. 20 to Nov. 30 Canisters KF-7 through 10 were in 4°C and uncontrolled humidity.

Nov. 30 12:00 PST (15:00 EST) They were removed and placed in their experimental configurations and put into a chamber under the following conditions:

<u>Temperature ($\pm 0.5^\circ\text{C}$)</u>		<u>Relative Humidity ($\pm 3\%$)</u>	
Nov. 30 - Dec. 12	21°C	Nov. 30 - Dec. 5	57%
Dec. 12 - Dec. 17	22°C	Dec. 5 - Dec. 12	60%
Dec. 17	23°C	Dec. 13 - Dec. 15	65%
Dec. 18 - Dec. 20	24°C	Dec. 15 - Dec. 17	60%
		Dec. 17 - Dec. 20	62%

Dec. 20 04:30 PST (07:30 EST) Canisters KF-7 through 10 were removed from the chamber and placed in the biotransporter. They were flown to N.Y. and brought to Stony Brook in the biotransporter by Mr. Robert Mah.

Temperature

approx. 4°C

21:00 EST The canisters were removed from the biotransporter at Stony Brook and placed in a 4°C refrigerator.

4°C

VII. Stony Brook Synchronous Control Flight (Canisters KF-11 through 14)

- Nov. 19 - Nov. 30 Canisters KF-11 through 14 were in a 4°C refrigerator (uncontrolled humidity) at Stony Brook. 4°C
- Nov. 30 - (15:00 EST) The canisters were removed from 4°C and placed in 22°C (uncontrolled humidity) in their experimental configurations. 22°C
- Dec. 20 (09:45 EST) The canisters were removed from 22°C and placed in a 4°C refrigerator. 4°C

VIII. Schedules of Observations

- Dec. 17 Petri dish #4 of canisters KF-1,2 & 3 was photographed and fixed with gluteraldehyde by A.D.K. in the U.S.S.R. M. Butenko fixed and ADK photographed dishes #3,7, and 9 from KF-1,2, and 3.
- Dec. 22 Naked-eye observations were made by F.R. Dutcher and E. Yim at Stony Brook of the dishes from canisters KF-1,2,3,7,8,9,10,11,12,13, and 14. All #1 dishes (uppermost in the canister) of these canisters were observed under a dissecting scope and a count of organized forms was made by F.R. Dutcher.
- Dec. 23 All the following dishes were photographed by E. Yim: #1 dish of KF-1,2, and 3; all #1,3,4,7, and 9 dishes of KF-7 through 14. Close-ups (with color film) were taken of material in all #1 dishes.
- Dec. 24 Dishes #3,7, and 9 of canisters KF-7 through 14 were fixed at Stony Brook for M. Butenko as per her instructions. Dish #4 of these canisters was fixed intact with the gluteraldehyde method. Dish #1 of canisters KF-1,2,3, and 7 through 14 was divided into halves; one-half was fixed with gluteraldehyde while the other half was kept at 4°C for later counting.
- Dec. 27 The fixed material was sent by A.D. Krikorian to Madame R.G. Butenko i.e. dishes #3,7, and 9 of KF-7 through 14.
- Dec. 23-Jan. 14 All remaining unfixed dishes (i.e. #2,5,6, and 8) of canisters KF-1,2,3,7 through 14 had a count of organized forms done by F.R. Dutcher.

Appendix C.

Table 1. Degree of Organization in Material from Canisters Exposed to Various Environments. Numbers refer to organized forms subjectively scored stages "1" to "4". Data represent the average number of individual forms counted per dish in a sample of 5 dishes.

Experimental Treatment	Stage 1	Stage 2	Stage 3	Stage 4	Average Total Number of Organized Forms/Dish
USSR Flight OG (KF-1)	1221 ± 336	336 ± 119	152 ± 65	94 ± 37	1803 ± 299
USSR Flight 1G (KF-2)	1131 ± 263	269 ± 45	173 ± 44	98 ± 26	1671 ± 239
USSR "Back-up" (KF-3)	1006 ± 250	184 ± 82	157 ± 112	96 ± 51	1443 ± 225
ARC Stationary Vertical (KF-7)	996 ± 188	214 ± 137	209 ± 36	161 ± 49	1580 ± 221
ARC Clinostat Vertical (KF-8)	1069 ± 152	169 ± 60	184 ± 64	171 ± 69	1593 ± 178
ARC Stationary Horizontal (KF-9)	996 ± 98	192 ± 97	173 ± 60	161 ± 60	1522 ± 122
ARC Clinostat Horizontal (KF-10)	1183 ± 249	186 ± 182	107 ± 32	90 ± 26	1566 ± 201
Stony Brook Stationary Vertical (KF-14)	1286 ± 250	109 ± 70	67 ± 14	65 ± 14	1527 ± 291
Stony Brook Clinostat Vertical (KF-12)	723 ± 247	86 ± 54	48 ± 32	45 ± 26	902 ± 230
Stony Brook Stationary Vertical (KF-13)	1223 ± 159	180 ± 84	116 ± 56	84 ± 40	1603 ± 140
Stony Brook Clinostat Horizontal (KF-11)	719 ± 213	59 ± 50	31 ± 7	25 ± 10	834 ± 238
Average number of each stage/dish	1048 ± 192	180 ± 77	129 ± 59	99 ± 48	1456 ± 304

HZE-PARTICLE DOSIMETRY*

D. D. Peterson, E. V. Benton, and M. Tran

Final Report

Technical Report No. 42

Department of Physics
University of San Francisco
San Francisco, California 94117

*Work sponsored by NASA-Ames Research Center, Moffett Field, California
under Contract NAS2-7069.

ABSTRACT

An experiment designed to measure high-LET particle radiation was one of four U.S. experiments flown aboard a Soviet, near-Earth orbiting satellite designated Cosmos 782 in November, 1975. The results derived from measurements in plastic nuclear track detectors include flux, integral LET spectrum, charge distribution, and stopping density of high-LET particles. One objective of this experiment was to determine the number of particles with $Z \geq 20$ stopping per unit volume. The value obtained aboard the 19.5 day mission was 0.9 cm^{-3} .

INTRODUCTION

A unique opportunity to fly a few U.S. experiments aboard a Russian Cosmos satellite was offered by the Russians in 1974. Ultimately four U.S. experiments were accepted aboard Cosmos 782 which was launched at 8:00 pm Moscow time on November 25, 1975. One of these experiments, proposed by our research group at the University of San Francisco, was designed to measure the high-LET particle radiation aboard the Cosmos satellite. One objective of this experiment, officially designated as experiment K103, was to determine the number of heavy particles with charge $Z \geq 20$ stopping in a one cubic centimeter volume. This information is necessary in order to assess the feasibility of performing U.S. experiments concerning heavy particle track effects in rodent brains to be carried out aboard future Russian Cosmos satellite flights.

The remainder of this report describes the methods and procedures used in this experiment and discusses the results obtained for the flux, integral LET spectrum, charge distribution, and stopping density of high-LET particle radiation aboard Cosmos 782.

METHODS AND PROCEDURES

The experimental methods and procedures used in the investigation are described in the following sections. They include flight information, high-LET particle detector, detector processing, track measurement, and data analysis.

A. Flight Information

On November 25, 1975 a joint American/Russian biological satellite, officially designated Cosmos 782, was launched from Russia at 8:00 pm Moscow

time. It was recovered on December 15, 1975 at 9:00 am corresponding to a total flight time of 19.5 days. The orbit inclination was 62.8 deg with apogee/perigee altitude of 405/226 km.

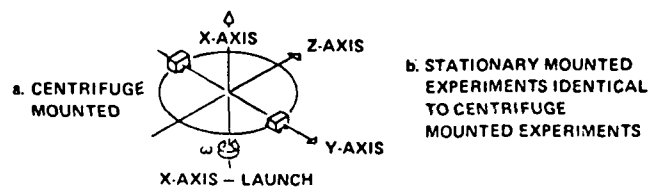
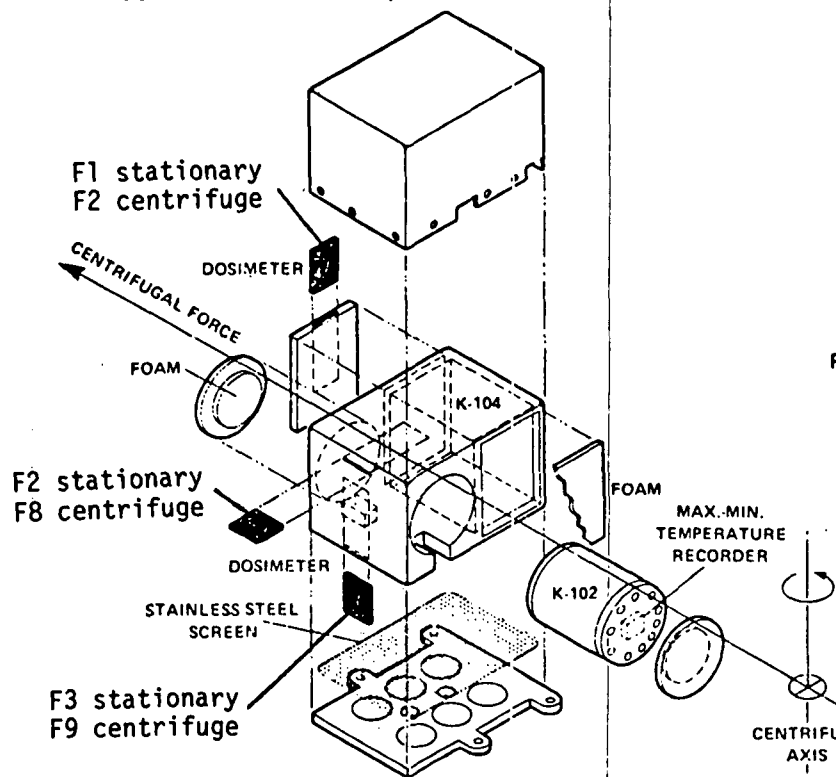
B. High-LET Particle Detector

Measurement of the high-LET particle radiation aboard Cosmos 782 was made with plastic nuclear track detectors.

Plastics used were cellulose nitrate (CN) and Lexan polycarbonate. The minimum LET value that can be detected with these plastics is $\sim 100 \text{ keV}/\mu\text{m} \cdot \text{Tissue}$.

Two different detector designs were used to make the measurements. One design, the thin detector, measured high-LET particle flux and integral LET spectrum. The other, the thick detector measured the charge spectrum. The thin detector consisted of seven thin plastic films layered together to form a stack. The stack was held together with tape. The surface area and thickness were, respectively, $5 \times 5 \text{ cm}^2$ and 1.5 mm. Aluminized mylar was wrapped around the stack to protect the plastics from any ultraviolet light exposure. Sets of three detectors, positioned so as to face along mutually orthogonal directions, were placed on containers housing experiments K101, K102, and K104 on the centrifuge. Sets of detectors were also placed similarly on these same experiments mounted in stationary locations. The positions of the thin detectors on experiments K101, K102, and K104 are shown in Fig. 1. A total of twelve thin detectors were flown aboard Cosmos 782. The thick detector consisted of seventy-five plastic films layered together to form a stack. The thickness and area were 2 cm and $9 \times 9 \text{ cm}^2$, respectively. Two thick detectors were placed inside Cosmos 782. Plastic films used in the detectors have thicknesses of $100 \mu\text{m}$ (CN) and $250 \mu\text{m}$ (Lexan).

CARROT CELL CULTURE FLIGHT HARDWARE
CONFIGURATION (K-102), WITH EXPERIMENT K-104



PLANT TUMOR GROWTH FLIGHT HARDWARE
CONFIGURATION (K-101)

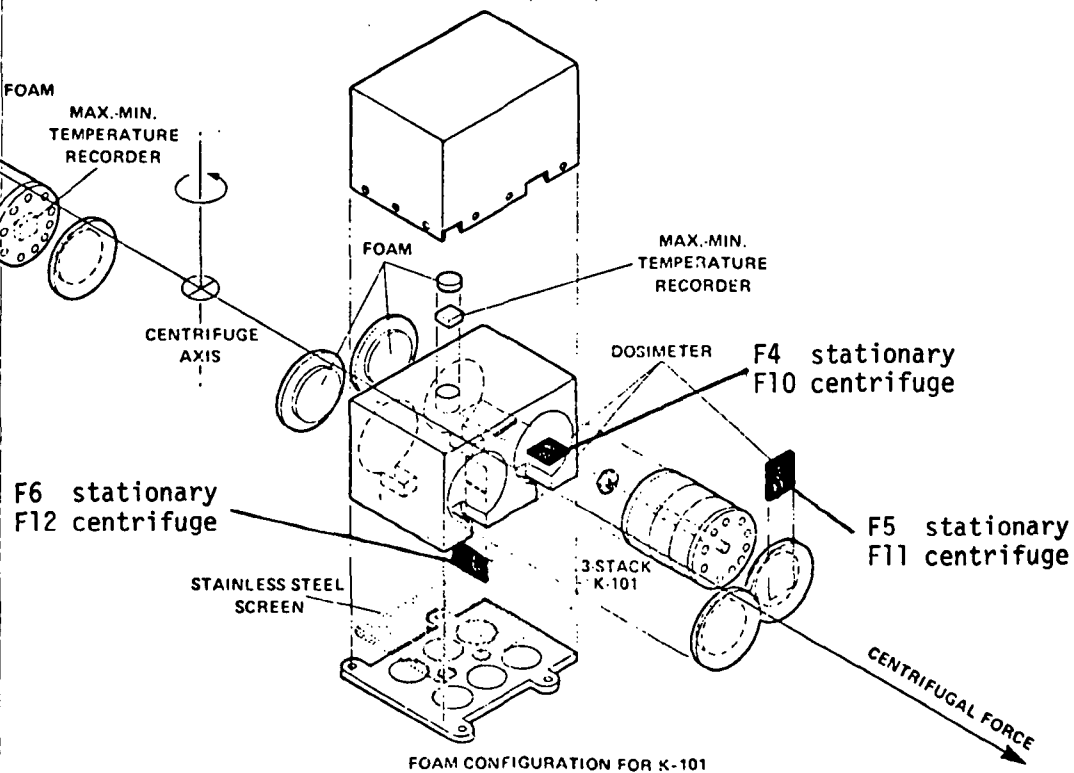


Figure 1.
Experiments K101, K102, and K104.

C. Detector Processing

Latent particle tracks in plastics are developed by chemically etching the exposed films. In this process, the damaged region along the path of the particle is preferentially attacked by the etchant. The resulting track appears as a hole in the plastic film whose size is determined by the duration of the development process.

A CN plastic film (layer #3) from each of the twelve thin detectors was developed in a temperature controlled etching bath. The etching temperature was $40.0 \pm 0.2^{\circ}\text{C}$, the etchant was 6.25N aqueous NaOH, and the etch duration was 18 hr. The etchant was mechanically stirred during the development. A Lexan film (layer #4) from thin detector F-7 was etched at 40°C in 6.25N NaOH + 0.05% Dowfax (a surfactant) for 96 hr. The etchant was saturated with Lexan etch products prior to the development. Seventy Lexan films from a thick stack were etched at 70°C in the saturated etchant for 55 hr. Plastics are rinsed for 15 minutes in hot running water following the development.

D. Track Measurement

Scanning and measurement of tracks in plastic films is done with high-resolution optical microscopes. The measured parameters of a track are its depth and projection. The precision in these measurements is of the order of $\pm 1 \mu\text{m}$. Track measurements are made typically at 1000X magnifications. Etched tracks normally have a diameter up to $\sim 20 \mu\text{m}$ at the surface of the plastic film. The track length may be $> 100 \mu\text{m}$.

E. Data Analysis

High-LET particle fluence ($\text{particles}/\text{cm}^2$), flux ($\text{particles}/\text{cm}^2 \cdot \text{s}$), integral number LET spectrum (flux of all particles with LET value greater than a specific LET value) and charge spectrum are calculated from track measure-

ment data. Track fluence and flux are gotten basically from track density and mission duration data. LET spectrum and charge spectrum are calculated using track measurement and calibration data. The LET value of a particle track is determined from the track etch rate in the plastic. The relationship, for etching conditions given above, is

$$\text{LET}_{350} = \frac{80.7 V_T^{0.316} \text{ in CN}}{767 V_T^{0.528} \text{ in Lexan}},$$

where LET_{350} is the restricted energy loss ($\text{keV}/\mu\text{m}$) and V_T is the track etch rate ($\mu\text{m}/\text{hr}$). Track formation in plastics follows LET_{350} (the subscript refers to the maximum energy in electron volts of secondary electrons included in calculating the restricted energy loss) rather than LET_{∞} (i.e. dE/dx). Conversions between LET_{350} in plastics and LET_{∞} in tissue (or water) can be made with the following relationships which are valid over a large range in these materials,

$$\text{LET}_{350}^{\text{CN}} = 1.16 \text{LET}_{350}^{\text{Lexan}}$$

and

$$\text{LET}_{\infty}^{\text{Tissue}} = 1.323 \text{LET}_{350}^{\text{CN}},$$

where LET values are in units of $\text{keV}/\mu\text{m}$. The integral LET spectrum follows from a summation of all particle tracks with LET value greater than some specific value. It is necessary to measure the change in LET along a particle trajectory in order to deduce the particle atomic number. Therefore, a thick stack of plastic films is required since the LET for most particles does not measurably change across the thickness of one plastic film. Measurement of particle LET and change in LET with range are sufficient, along with calibration data, to determine particle atomic number.

RESULTS AND DISCUSSION

The results to be presented and discussed include flux, integral LET spectrum, charge distribution, and stopping density of high-LET radiation aboard Cosmos 782. They provide baseline data needed by U.S. scientists to assess feasibility of investigations concerning radiobiological effects of high-LET particle radiation in future Cosmos flights.

A. Flux

Table 1 presents results derived from twelve thin detectors located on containers housing experiments K101, K102, and K104. The first and second columns designate, respectively, the detector number and detector location. The third, fourth, and fifth columns list for each detector, respectively, observed tracks, fluence, and flux. The fluence and flux values correspond to particles having $LET_{350} \geq 80 \text{ keV}/\mu\text{m}\cdot\text{CN}$ ($LET_{\infty} \geq 105 \text{ keV}/\mu\text{m}\cdot\text{Tissue}$) and range $\geq 100 \mu\text{m}\cdot\text{CN}$.

The average flux of $4.05 \pm 0.70 \text{ particles}/\text{cm}^2\cdot\text{day}$ inside Cosmos 782 is 21% larger than the flux recorded for Skylab (SL4) astronauts ($3.35 \pm 0.36 \text{ particles}/\text{cm}^2\cdot\text{day}$).⁽²⁾ This result is expected due primarily to the higher inclination orbit (62.8 deg) of Cosmos 782 compared with Skylab (50 deg). High-inclination orbits are subject to lower cutoff rigidities and consequently receive higher particle flux compared with low-inclination orbits.

Differences in particle flux values within sets of detectors (e.g. F1, F2, and F3) and between sets of detectors reflect shielding distribution variances. The plastic detector records incident particles most efficiently at small angles of incidence. At large angles the efficiency decreases rapidly. Each of the three detectors in a set face along mutually perpendicular directions, may view different shielding thicknesses, and may, therefore, record

TABLE 1

Sample	Location	Observed Tracks ^a	Fluence ^b (tracks/cm ²)	Flux ^{b,c} (tracks/cm ² ·day)
F1/3CN	K102, K104 stationary	346 ^d	86.5	4.44
F2/3CN		293 ^d	73.3	3.76
F3/3CN		260 ^d	65.0	3.33
F4/3CN	K101 stationary	132	66.0	3.38
F5/3CN		158	79.0	4.05
F6/3CN		145	72.5	3.72
F7/3CN	K102, K104 centrifuge	201	101	5.15
F8/3CN		128	64.0	3.28
F9/3CN		194	97.0	4.97
F10/3CN	K101 centrifuge	145	72.5	3.72
F11/3CN		199	99.5	5.10
F12/3CN		142	71.0	3.64

average = 4.05 ± 0.70

^ain 2 cm² area.

^bcorresponds to LET₃₅₀ ≥ 80 keV/μm·CN (≥105 keV/μm·Tissue) and >100 μm range.

^c19.5 day mission.

^din 4 cm² area.

different particle flux than the other detectors corresponding to the amount of shielding in the direction the detector faces. Shielding reduces high-LET particle flux.

The averaging effect of the centrifuge regarding shielding distribution variations is readily apparent in detectors F7, F8, and F9. Detectors F7 and F9 were mounted with their surfaces parallel to the centrifuge axis of spin (see Fig. 1). They viewed, therefore, all angles normal to the spin axis. A flux of ~ 5 particles/cm² day was recorded in F7 and F9. Detector F8 was mounted with its surface perpendicular to the spin axis and viewed incident particles at a fixed solid angle of incidence. The lower flux of ~ 3.3 particles/cm²·day is therefore expected in detector F8.

B. Integral LET Spectrum

The LET spectrum of high-LET particles is an important dosimetric parameter required for assessing potential radiobiological hazard produced by these particles in spaceflight. Plastic dosimeters are ideally suited to measure this parameter. In practice, measurements in a few layers of plastic film are required to determine the spectrum.

Figure 2 is a graph of the integral number density of high-LET particles versus LET value. The integral LET spectrum measured in thin dosimeter F7 is shown in Fig. 2. For comparison, spectra measured in the Skylab mission, (2, 4) in the Apollo 17 lunar mission, (1) and calculated for cosmic-ray Fe nuclei (2) are also displayed in Fig. 2. These curves indicate that the magnitude and slope of the integral LET spectrum are proportional to the amount of shielding. Generally, for near-Earth missions, high-inclination orbits receive a higher flux of particles due to a lower cutoff rigidity than low-inclination orbits. Lunar missions normally receive higher exposures than near-Earth missions due to absence of shielding of particles by the Earth. Cosmos 782 orbit inclina-

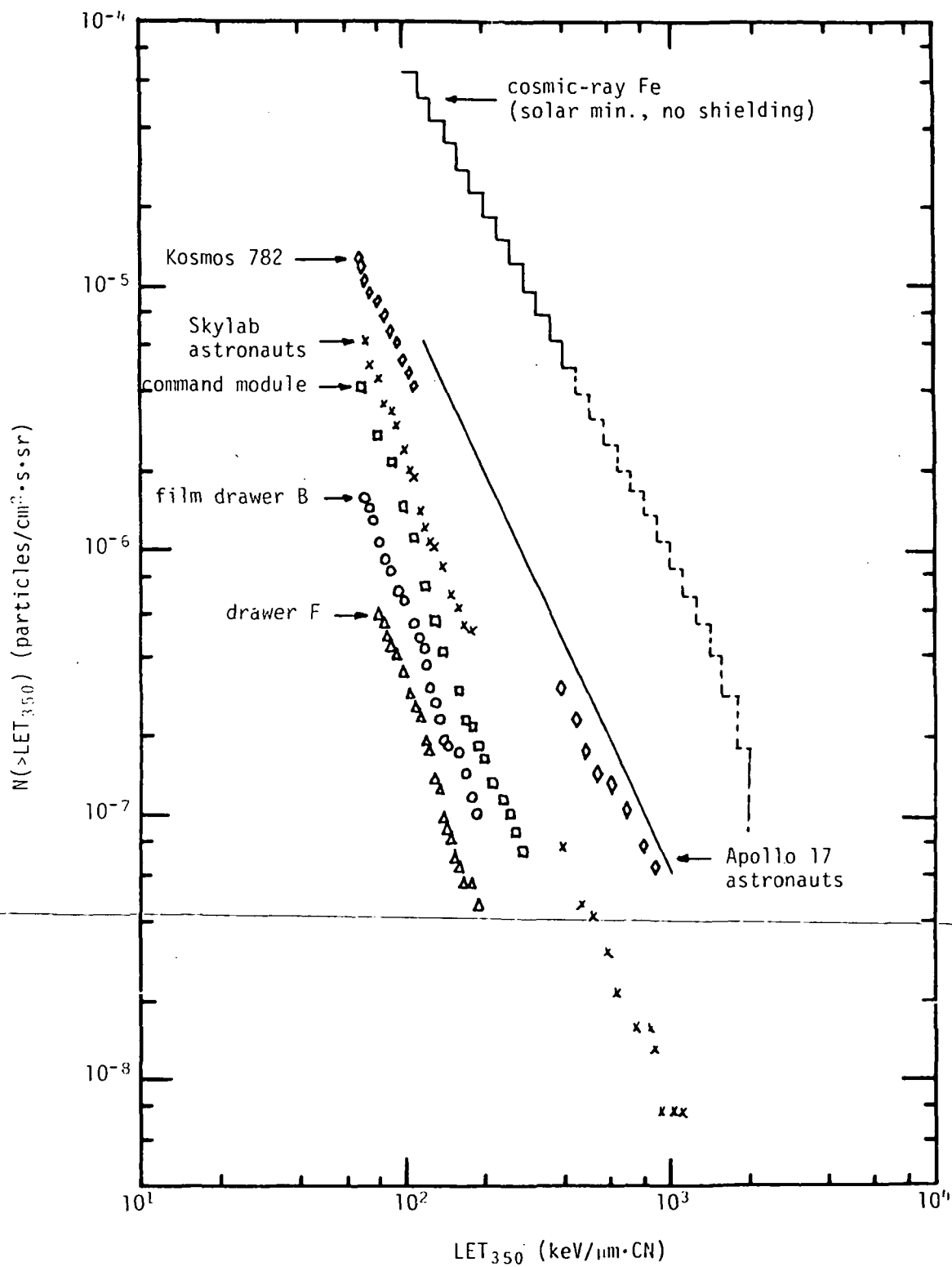


Figure 2
Integral number LET spectrum measured aboard spacecraft
with plastic nuclear track detectors.

tion was ~ 63 deg; Skylab was 50 deg. The altitude of Cosmos 782 was 405/226 km (apogee/perigee) and 435 km for Skylab. Low-altitude orbits receive a smaller particle flux due to increased absorption of particles in the atmosphere compared with high orbits. The high-inclination orbit though is the main reason for the larger flux of high-LET particles aboard Cosmos 782 compared with Skylab.

The main feature of all spectra in Fig. 2 is the sharp decrease in particle flux toward large LET values. The number expected at large LET values, ≥ 1000 keV/ μ m, is therefore extremely small. The curves in Fig. 2 indicate a few per year.

C. Charge Distribution

The low sensitivity of plastics is an advantage which allows selective measurement of relatively small numbers of high-charge particles under the conditions of a large background of low-charge, lightly ionizing, particles. In Cosmos 782, the charge distribution of high-charge particles ($10 \leq Z \leq 26$) was determined from track measurements in a multilayered, thick stack of plastic films.

The charge distribution measured inside Cosmos 782 is shown in the graph in Fig. 3. It is a plot of the observed number of stopping particles over the interval $10 \leq Z \leq 26$. Charge values have a precision of ± 0.5 . The charge identification scheme used is based on the maximum particle range for which holes are etched completely through the plastic films. The data result from scanning a 5×5 cm² area, 1.85 cm thick, stack of 73 plastic films.

The charge distribution in Fig. 3 shows many interesting features. First, the number of ^{26}Fe nuclei is unexpectedly low compared with other charges. This may partly be explained as resulting from fragmentation in the spacecraft walls, and partly caused by reduced detecting efficiency. A thicker stack would

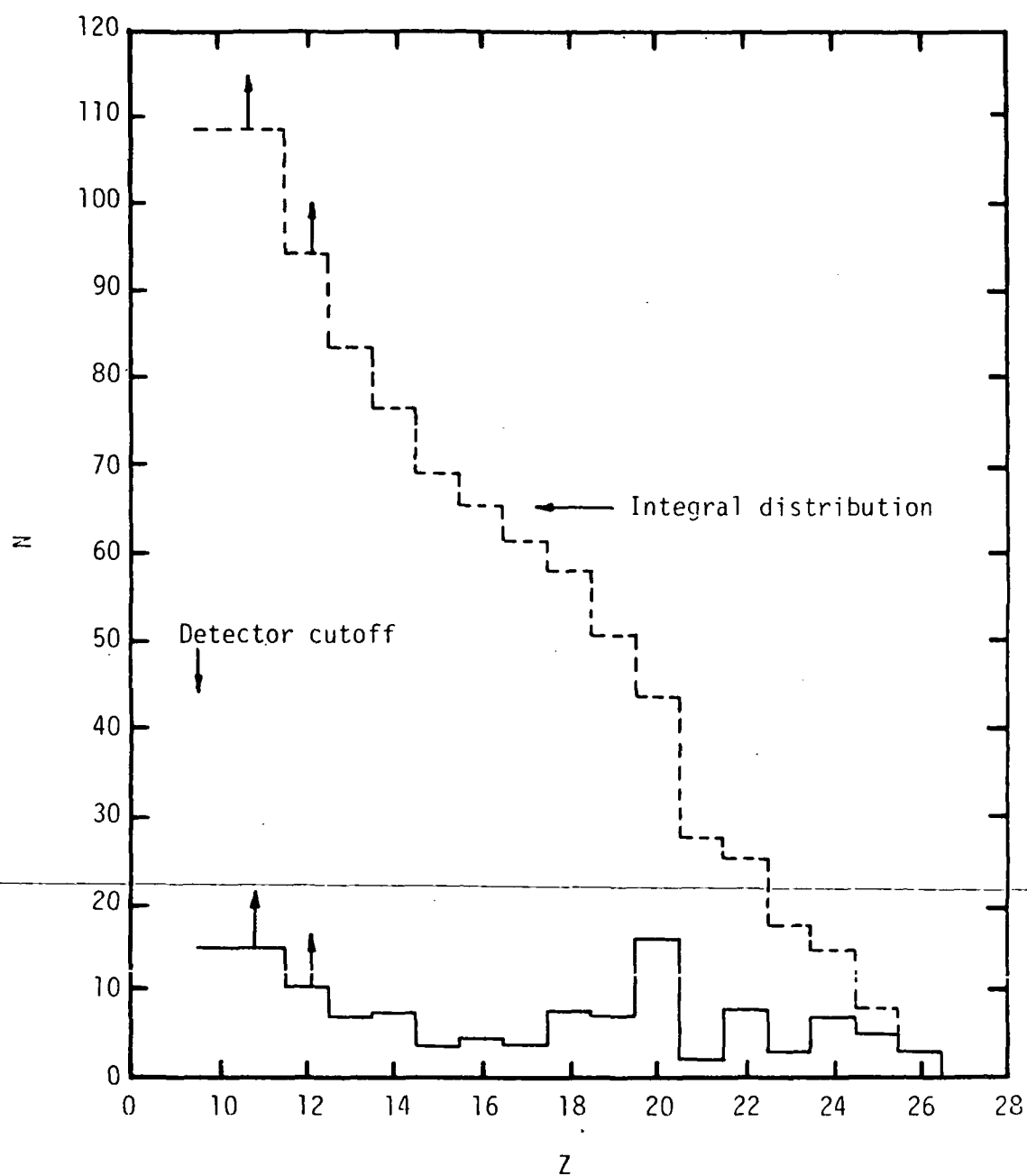


Figure 3
Charge distribution of heavy particles stopping in a thick
stack of plastic films aboard Cosmos 782.

have a higher efficiency. The relatively higher number of ^{24}Cr and ^{22}Ti compared with ^{26}Fe supports the idea of fragmentation of ^{26}Fe into these nuclei. In free space, behind no shielding, ^{26}Fe is more abundant than either ^{24}Cr or ^{22}Ti .⁽³⁾ Including the scanning efficiency for ^{26}Fe tracks, which is ~ 0.7 , the number of ^{26}Fe nuclei is still smaller than either ^{24}Cr or ^{22}Ti . In the region $Z \geq 20$, the most abundant particles are nuclei of ^{20}Ca . The abundance ratio of calcium to iron corrected for scanning efficiency has a value of ~ 3 . The minimum charge value recorded was $Z \approx 10$. Charges were not resolved over the interval $10 \leq Z \leq 16$. Also, as indicated in Fig. 3, the true number of $10 \leq Z \leq 16$ particles stopping in the detector ($5 \times 5 \text{ cm}^2 \times 1.85 \text{ cm}$) exceeds the value indicated. This is because a complete scan of the total thickness was not carried out for these events. All stopping events $Z \geq 16$ were located and measured in the stack dimensions given above. The dashed line in Fig. 3 shows the integral number of stopping events in the stack.

A layer of cellulose nitrate plastic (layer no. 39) positioned at the center of the thick stack registered fluence and flux values, respectively, of $67.5 \text{ particles/cm}^2$ and $3.46 \text{ particles/cm}^2\cdot\text{day}$. These values correspond to particles with $\text{LET}_{\infty} \geq 100 \text{ keV}/\mu\text{m}\cdot\text{Tissue}$ and residual range $\geq 100 \mu\text{m}$. This value is lower by 14.6% than the average flux measured by the twelve thin dosimeters. The lowest value measured by the thin dosimeter is, however, $3.28 \text{ particles/cm}^2\cdot\text{day}$ in no. F8 (see Table 1).

D. Particle Stopping Density

The stopping density of $Z \geq 20$ nuclei inside Cosmos 782 is determined on the basis of the number of stopping particles in the thick stack. This result is used in the estimation of the number of stopping $Z \geq 20$ particles in the brain of a small rodent (gerbil) in order to assess the outcome of a U.S. experiment planned to fly aboard a Cosmos biological satellite in 1977. The objective of the planned experiment is to locate lesions in the brain produced

by individual high-LET particles. Particle charge, trajectory, and stopping point in the brain will be determined through particle track measurements made in plastic detectors implanted beneath the rodent's scalp.

Table 2 displays the pertinent data obtained in the analysis of the thick stack. The number stopping values listed in column two correspond to the results from consecutive ten-layer intervals of plastic films throughout the stack and include all particles traveling upward and downward. The number traveling downward was approximately three times the number traveling upward through the stack. The stopping density given in column three is calculated for each ten-layer interval. Values are not given for intervals near the top or bottom of the stack since detector efficiency is low in these regions. The average particle stopping density from Table 2 is 0.06 ± 0.03 particles/cm³·day.

E. Expected Results for Gerbil-Brain Monitor — Cosmos '77

Based on the results of this investigation the expected number of particle tracks per gerbil-brain monitor in the Cosmos '77 mission is 160 ± 28 particles per monitor. This value assumes a 2 cm² area monitor and a 20 day mission duration. This represents an order of magnitude greater fluence than was registered in the pocket mouse brain monitors aboard the Apollo 17 lunar mission. The characteristics of these 160 particles are listed in Table 3. The first, second, and third columns show, respectively, that all 160 particles have $LET_{\infty} \geq 100$ keV/μm·T, 6 have ≥ 500 keV/μm·T, and 2 have ≥ 100 keV/μm·T. The 160 particles are distributed in charge according to the values given in columns four through seven. The residual range of particles corresponding to the various charge groups is also given in columns four through seven. Column eight gives the most important data regarding the number of stopping $Z \geq 20$ particles in the gerbil brain. It shows that there will be ~1 particle with $Z \geq 20$ detected in the monitor, out of the total 160 particles, which will stop within 0.2–10 mm range beyond the monitor (i.e.

TABLE 2

Layer Interval	Number Stopping ^a	Stopping Density ^b (particles/cm ³ ·day)
1-10	1	
11-20	11	0.09
21-30	9	0.07
31-40	5	0.04
41-50	11	0.09
51-60	4	0.03
61-70	5	0.04
71-73	3	

^acorresponds to particles $Z \geq 20$; 5×5 cm² area scanned.

^b19.5 day mission, 1 layer = 0.025 cm.

TABLE 3. CHARACTERISTICS OF THE 160 PARTICLE TRACKS EXPECTED PER GERBIL-BRAIN MONITOR
(2 cm² area) IN A 1977 COSMOS FLIGHT (20 days)

Number of Tracks ^(a)			Charge Group ^(b)					Direction ^(c)	
LET _∞ ≥ 100	≥ 500	≥ 1000 keV/μm·Tissue	6-9	10-14	15-25	26-28	≥20	Down	Up
160	6	2	7	21	31	100	~1 ^(c)	75%	25%
Residual Range (mm)			0.3-1.9	0.2-9.9	0.2-88	0.2->200	0.2-10		

(a) Based on results derived from measurements in 12 plastic dosimeters exposed aboard Cosmos 782 (1975).

(b) Based on calculations, see D. D. Peterson and E. V. Benton, Health Physics **29**, 125-130 (1975).

(c) Based on results derived from measurements in a stack of 73 Lexan plastic films exposed aboard Cosmos 782.

the approximate depth region of the gerbil brain). The ninth column shows that 75% of the particles recorded in the monitor will be traveling in one direction, downward for example, and 25% will be traveling in the opposite direction.

Summarizing these results then, the number of detectable, stopping, $Z \geq 20$ nuclei in the gerbil brain is at most ~1 particle in a 19.5 day 1977 Cosmos mission.

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KILLFISH DEVELOPMENT IN ZERO-G ON COSMOS 782:

Fundulus Experiment K-104

by

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INTRODUCTION

The Kosmos K-104 Fundulus embryogenesis experiment was the third in a series of experiments to assess the possible effects of the space environment upon developing organisms. The experiment evolved from the Apollo Soyuz Test Project (ASTP) experiment MA-161. The major refinement lay in the incorporation of a 1-gravity onboard control treatment through the use of an onboard centrifuge. Other notable features were the employment of a low temperature transport system for retardation of development during shipment to the Soviet launch site and an adjunct Soviet experiment with clinostatted embryos.

EXPERIMENT BACKGROUND

The experimental organism was Fundulus heteroclitus (Walbaum) a small shallow-water minnow common to the Atlantic coast of North America. The Beaufort, North Carolina, strain employed in these experiments produces an egg having a transparent chorion thus allowing observation of development from first cleavage through hatching. The chorion is quite tough and the egg may be manipulated extensively without undue stress on the developing embryo. Because the biology and developmental relationships of the Fundulus are relatively well known (Armstrong & Child, 1965; Oppenheimer, 1937), and because of its physical attributes which allowed aseptic culture, the organism was used as a test organism at the NASA Lyndon B. Johnson Space Center (JSC) during the lunar quarantine. Subsequently the methodology

for continuous laboratory production of fertile eggs was developed in this laboratory and the accumulation of a data base for flight experimentation was initiated.

The initial Fundulus experiment was flown on the Skylab 3 mission. The experimental design was quite preliminary in form. The flight package consisted of a plastic bag containing 2 juvenile fish in a compartment with 250 milliliters of synthetic seawater (21 parts per thousand dissolved solids) and 50 fertilized eggs (5 days past fertilization) in a separate compartment with 50 milliliters of seawater. Initially, the juveniles exhibited obvious disorientation reactions (swimming rapidly in loops and circles), but over a period of several days in orbit, they gradually adapted to the weightless environment and to dependence on visual cues for orientation. In this state of adaptation, the locker door surface to which the plastic aquarium was attached served as "down". Adaptation was not complete, however, and occasional disoriented swimming activity occurred. Nearly all the 50 eggs hatched in space, and because of a delay in hatching of the flight fish, several returned alive to Earth. However, a series of unfortunate events resulted in the death of these animals shortly after return. The space-hatched Fundulus fry exhibited no observable tendency toward disoriented swimming activity, and their apparent dependence on visual

orientation cues both onboard the Skylab spacecraft and on the recovery ship suggested the possible absence of vestibular input. Preservation of the returned hatchlings was insufficient to prevent deterioration and did not permit definite conclusions regarding the condition of the vestibular system.

The second experiment in the series was the MA-161 experiment flown on the 9-day Apollo Soyuz Test Project mission to confirm and extend the observations of the Skylab experiment (Scheld et al., 1975, 1976). The experiment package consisted of two parts: a series of staged embryos in five individual compartments of a polyethylene bag and a series of preconditioned juvenile fish in a similar bag. Embryos at 32, 66, 128, 216, and 336 hours after fertilization at the time of launch were chosen to represent key stages of development; development occurred at a constant temperature of 295°K (22°C). Juvenile fish were reared from hatching for 21 days in specific visual environments. Experiment packages were mounted on the docking module wall and photographed periodically during the mission to record the swimming activity of the fish and the condition of the eggs. At splashdown and at selected times thereafter, juveniles and hatchlings were tested to assess normalcy of vestibular function and samples were fixed for microscopic examination to assess normalcy of anatomical development.

Juvenile fish in a null-gravity environment exhibited looping swimming activity similar to that observed during the Skylab 3 mission. Hatchlings from the 336-hour egg stage also were observed to loop. At splashdown, both juveniles and hatchlings exhibited a typical diving response suggesting relatively normal vestibular function. The juveniles exhibited swimming patterns suggestive of abnormal swim bladders. Rotating drum tests confirmed that no radical changes in vestibular function had occurred, and no significant differences were found in subsequent light orientation tests. Tests of geotactic response in fish after 6 months or more of development suggested a tendency for the 32-hr flight fish to spend a significantly greater portion of their time in the upper portion of the test apparatus. Other treatment groups exhibited no statistically significant tendency, but in all groups tested there was evidence that the flight fish might be more sensitive to environmental factors.

Extensive light and electron microscopic examination of flight and control materials have revealed no significant differences in the embryological development of the central nervous system, peripheral vestibular apparatus, the eye or the cardiovascular system, of any of the animals examined thus far. The otoliths of flight juveniles were compared by scanning electron microscopy with otoliths from control animals maintained on Earth. The medial surfaces of the sagittae are characteristically grooved and sculptured; the lateral surfaces are smooth with concentric "growth rings" and "crystalline" projections in the groove in larger fish. None of the scanning electron microscopic examinations revealed recognizable differences in size, shape, or surface structure between flight and control juveniles. Histological and ultrastructural analyses of all age categories revealed that the peripheral vestibular system developed fully with complete expression

of the maculae of the sacculus and utriculus. The statoliths of the sacculus and utriculus followed normal sequence, and the lagenal statolith initiated development between posthatching days 4 and 6 in all groups. Except for a decrease in the extent and content of the vitreous cavity, ocular development was judged to be normal. Ultrastructural analyses of the developing statoliths and sensory maculae have revealed no differences between matched groups of animals. Both sustentacular and hair cells followed normal developmental parameters. Central projections and vestibular ganglia contained normal cellular complement and projection configuration. Cartilage development and calcification was identical in flight and control categories. Preliminary analysis of the specimens treated with tritiated thymidine at selected postsplashdown periods has also failed to reveal any significant alterations in the patterns of cellular proliferation within the vestibular, ocular, or central nervous systems of the oldest stages flown. Cells of the ependyma and cortical neuroepithelium, the planum semilunatum of the maculae, and the ora serrata of retina were heavily and consistently labeled in all groups.

EXPERIMENT DESCRIPTION

The main experiment package of the Cosmos K-104 experiment was based upon the design of the ASTP experiment and modified only to the extent necessary to conform to the new requirements and constraints of the Soviet spacecraft, launch/recovery procedures, and flight hardware. Each experimental unit consisted of a polyethylene bag containing 50 embryos of a given age and 23 ml sterile filtered 21% Instant Ocean. An experimental treatment contained 2 units each of five different embryo age groups as follows;

<u>Unit</u>	<u>Calculated Launch Age</u>	<u>Average State of Development</u>
1	32 hr	Mid-to-late gastrula
2	42 hr	Tubular profile of membranous labyrinth apparent. No evidence of statolith precursors
3	66 hr	Appearance of statolith precursors
4	88 hr	Initiation of ventral elongation of medial sensory patch. Initial elaboration of semi-circular canals
.5	128 hr	Statoliths formed but without definitive margins

Age was based upon development time at 295 K (22 C). Each treatment group thus contained 100 embryos of each of the 5 different nominal development stages for a total of 500 eggs.

Experimental treatments were as follows:

K-1: Flight treatment, null-gravity-(stationary-mounted). Five hundred eggs as indicated above were fertilized, allowed to develop and then packaged, chilled, transported and flown as indicated in Table 1.

K-2: Flight treatment, one-gravity onboard control (centrifuge mounted). Eggs were treated identically with K-1.

K-3: Ground Control 1. Eggs were prepared and transported with K-1 and 2. At the time indicated in Table 1 the experiment package was mounted on a 5 rpm tube rotator and subjected to slow rotation (tumbling) in order to simulate the uniform dispersion of eggs in null-gravity.

K-4: Ground Control II. Eggs were prepared and transported with K-3. The flight package was allowed to remain in a stationary position. Ten eggs

Table 1

Environmental Conditions Encountered by Cosmos Experimental Embryos

Treat- ment Number	22°C Prechill Incubation Time for Embryos					Temperature/Treatment Profile					
	128 hr	88 hr	66 hr	44 hr	32 hr	Chill Cycle	Transport Cycle 10°C	Warm-up	Flight	Recovery	
K-1	108	68	46	24	12	1.75 hrs. (18°C-10°C)	65 hrs (drop to 8.6° for 4-5 hrs)	8 hrs 21°C	19½ day 21° 0-G	52 hrs 10°C	
K-2	"	"	"	"	"	"	"	"	" Centrifuge	"	
K-3	"	"	"	"	"	"	81 hrs (Tempera- ture stable)	4 hrs 20°C	19½ days 21°C Rotated Moscow	14 hrs 21°C	
K-4	"	"	"	"	"	"	"	"	" Stationary Moscow	"	
K-5	"	"	"	"	"	"	90 hrs	3 hrs 22°C	20 days 22°C USA Rotated	24 hrs 10°C	
K-6							Continuous development at 22°C until hatching				

were removed from each development group and fixed at time of launch to provide a reference point for gauging the developmental stage at time of null-gravity exposure.

K-5: Ground Control U.S. Eggs were prepared identically with K-1 through K-4; transport was simulated and the flight package was mounted as for K-3.

K-6: Dish Control. Eggs were fertilized as for K-1 through K-5, but were allowed to continue development until hatching under standard environmental conditions in petri dishes.

EXPERIMENT HARDWARE

The experiment hardware consisted of four major items: the egg bags, the experiment container, the low temperature transporter and the outer flight container supplied by the Soviet side. The egg bag configuration was based upon that of the ASTP experiment. The same 2.6 mil clean room polyethylene was employed in fabrication but individual packets were used instead of the string of connected packets of the ASTP flight package. Dimensions of the flattened bags were 7.0 cm wide by 8.4 cm long inside the 2.5 mm heat seals. A double width heat seal was employed for final closure of the bag.

The experiment container was a two-chambered, machined aluminum, cuboidal case of 8.9 x 9.5 x 9.8 cm outside dimensions. Five egg bags were packed into each chamber sandwiched between thin perforated sheets of Pyrel(TM) foam. The case was closed by a perforated plate held in place by four Allen head screws.

The low temperature transporter consisted of an insulated aluminum case, approximate dimensions 35 x 40 x 45 cm with a central aluminum compartment for containment of the samples. The compartment was wound with heater wire. Current to the heater was furnished by dry cell batteries through a thermostatic control circuit. Between the sample chamber and the outside case, ~~separated from both by layers of foam insulation, were flat containers of~~ ice. The chilling capacity was supplied by the ice, restricted by the layer of insulation. This chilling effect was bucked by the heater circuit on the sample chamber. In operation this system allowed maintenance of $10\text{ C} \pm 0.2$ for a 72-hour period.

The Soviet supplied flight container was a sheet metal box fastened to a machined baseplate by means of screws. Screw holes in the baseplate mated with attachment points in the Soviet spacecraft. In operation the K-104 experiment container shared the flight container with another U.S. experiment container - the carrot cell experiment K-102. Both were held in place and protected by a preformed Pyrel foam filler.

DATA COLLECTION

The primary data yielded by the experiment was in the form of fixed material for light and electron microscopic analysis. Fixation was in the cold (ice bath) in Kalt-Tandler (Keefe, 1973) fixative. Samples were fixed at recovery and at intervals up to 30 days following recovery. The primary point of interest in all examinations was the vestibular and other sensory regions.

Postflight observation and testing were carried out to detect possible differences in orientation behavior attributable to the null-gravity exposure during development. The tests employed, light orientation, rotating striped drum and geotaxis test, have been described elsewhere (Scheld et al., 1975; Scheld et al., 1976) as they were employed for ASTP postflight testing. These same tests were used without significant modification.

EXPERIMENT EXECUTION

The basic procedure for preparation of fertilized eggs followed that of the ASTP experiment (Scheld et al., 1975). Specific timing for this experiment is indicated in Table 1. Eggs fertilized at the appropriate times prior to launch were allowed to develop to known stages of development and then sealed in the plastic packets and chilled to 10°C to retard further development during transport to the launch site. For planning purposes it was assumed that elapsed time of chilled transport would be 72 hours and time from rewarming and loading of flight package to launch would be 8 hours. Progress of development at 10°C for 72 hours was calculated to be equivalent to 12 hours at standard temperature of 22°C. Fertilization of eggs was thus scheduled to produce embryos that at the time of chilling would be 20 hours younger than the expected launch ages.

Slow chilling of the eggs was effected by placing the sealed plastic bags in plastic beakers containing 1000 ml of water and allowing these to chill to 10°C in a 6°C incubator. The chilled bags were then quickly transferred to the equilibrated 10°C transporter and packed for shipment.

Transport and launch of the flight package proceeded without incident. In Moscow at approximately 36 hours before launch, control eggs were removed to a separate transporter and held in Moscow for treatment, while flight eggs were taken to the launch site. As indicated in Table 1, eggs were removed from the transporters a few hours prior to launch, loaded into the experiment hardware and then placed onboard the spacecraft.

At recovery flight-treated packets were removed from the flight containers, marked and then placed in the 10°C transporter for shipment to the Moscow laboratory. Ground controls were not subjected to this chilling treatment because access to these materials was delayed by airline scheduling, until nearly 1-1/2 days after recovery and chilling of the flight materials had occurred. This deviation was considered to be justified in light of baseline laboratory tests which have indicated that chilling has no harmful effect and because a chilling treatment of control eggs would have caused a considerable delay and complication of the recovery procedure.

An adjunct experiment carried out by Soviet investigators considered the effects of clinostatting upon development. Eggs were fertilized and prepared by the standard procedures to be chilled and shipped 10 days prior to launch. Development, at the time of chilling for shipment, had proceeded for 12 hours, 36 hours and 66 hours, respectively, in three separate sets of 300 eggs each. Upon arrival in the Soviet Union, eggs from the three age groups were randomly divided into treatment and control groups for clinostat exposure. Periodically during the course of development, samples were removed and fixed for microscopic examination. At approximately 36 hours prior to launch, subgroups of the treatments were chilled, transported, and launched with the eggs of the main experiment. Results of these experiments are summarized in Krasnov (1976).

RESULTS AND DISCUSSION

Recovery Activities

The experiment was carried out according to the timing indicated in Table 1 and in the "Experiment Execution" section of this report. Upon opening of the experiment packages severe retardation and high anomaly rates were apparent in all treatments except the Houston Standard Dish Control. The originally planned sampling and testing schedules were modified to allow for the slower hatching rates, and reduced numbers of samples. All eggs were examined under a binocular microscope and counts of normal (Table 2), abnormal (with types of abnormality indicated) and dead were recorded. Following microscopic examination, all eggs were shaken for 20 minutes on a rotary shaker and then examined for hatching 20 minutes or longer after the end of the shaker period. Except for the time in transit from the USSR, this routine was followed until all normal eggs had hatched.

Significant numbers of hatchlings did not emerge until the third and fourth days postflight. All morphologically normal hatchlings exhibited a typical fright-diving response and there were no apparent differences among the treatments with respect to diving response. No changes in orientation were noted in response to unidirectional light through the sides or bottoms of the containers. Beginning on the third day post-recovery, motion picture recordings were made of normal and disturbed swimming activity, geotactic response and orientation in a rotating striped drum.

Table 2

Normal Hatchlings Record from the Kosmos Mission*

Treatment	<u>Age</u>				
	32 hrs.	44 hrs.	66 hrs.	88 hrs.	128 hrs
K-1 (flight stationary)	6	4	1	14	38
K-2 (flight centrifuge)	4	10	6	11	27
K-3 (ground rotated)	1	1	0	0	1
K-4 (ground stationary)	0	2	0	0	25
K-5 (USA rotated)	0	0	0	0	0
K-6 (USA dish)	99	97	100	97	98

*Not corrected for hatchlings from any eggs given to Soviet investigators. All treatments contained 100 eggs except K-4 which contained 90.

Samples of hatchlings and eggs were fixed for microscopic examination on the fourth, seventh, and thirtieth days postflight. Major sampling was from the population of anomalous hatchlings. Only representative samples were taken from the apparently normal population. All samples were post-fixed with osmium and embedded in Epon plastic for sectioning.

Behavioral Testing

It is now a virtual certainty that development in weightlessness from the earliest exposure achievable under the current experimental constraints, or the additional tenure in space for 8-14 days past time of theoretical full embryonic development has no radical effect upon vestibular function. Further, it has become obvious from all observations thus far that alterations in behavior, if they exist, will be distinguishable only by relatively high-resolution quantitative techniques.

Preliminary investigations (Scheld et al., 1976) of geotactic response in maturing (6-8 months old) ASTP hatchlings have shown a statistically significant difference in tendency of 32-hr hatchlings, only, to swim predominantly in the upper half of the test apparatus. There was also some indication that flight fish in general were more sensitive to their environment. The significance of such subtle changes is difficult to assess without further data; the preliminary results were not confirmed because of loss of the flight animals in a laboratory mishap.

Histological Examination

Since all of the animals flown on this flight were impacted by a non-spaceflight factor that caused a 90-95% anomaly production in both flight and ground control groups (except open-dish control group), microscopic studies have been restricted to the 5-10% of the specimens adjudged to be grossly normal. Additional control specimens were obtained from earlier studies flown on the ASTP mission. In the present material lesions of various regions of the CNS (predominantly diencephalic) indicate a less than normal overall process of development. These lesions have been observed in all groups except the open dish controls and were never encountered in specimens derived from earlier control groups.

The peripheral vestibular apparatus develops in both normal time and proper sequence. Lateral, anterior and posterior semicircular canals originate from the closed otocysts in usual sequence (figs. 3, 4, 6, 9 and 11). The utricular and saccular maculae originate from a common primordium and follow tubular rotation to become fully differentiated. Both utricular (figs. 6, 9 and 10) and saccular (figs. 6 and 11) maculae frequently demonstrate "darkcells" (fig. 9). No differences were observed in the distribution or frequency of such cells between age-matched flighted and ground control specimens.

The sensory maculae develop hair cells (figs. 10 and 13) capped with a single kinocilium surrounded with 21 to 24 microvilli in normal sequence. The pairing basal body in the apex of each hair cell (see B in fig. 13) gives rise to a system of rootlets (see r in fig. 13) that extend into the basal region of each cell. The apical terminal web region of each hair cell and the basal synaptic regions differentiated in normal time sequence.

The otolithic membrane (OM) appears to be derived by a dual system of cellular secretion in this species: (1) a process of apocrine secretion in which apical portions of sustentacular cells are released into the otolithic membrane proper (see CV and V in fig. 13); and, (2) a separate discharge of secretory materials derived from the extensive rough endoplasmic reticulum (see R in fig. 13) and involving a single large Golgi complex. These observations suggest that there are either two populations of sustentacular cells or two states of the same cell type responsible for the initial elaboration of the otolithic membrane.

Both the utricular (Lapillus) and the saccular (Sagitta) statoliths condensed in concentric layers around an apparent initiation site which remains visible as a metachromatic dense granule(s) (arrows in figs. 9, 10 and 11). Appositional growth continued both in flight and upon recovery prior to sacrifice with no apparent alterations in the rates of accretion of statolithic substance. Concretion rings displaying typical metachromasia (arrow in fig. 11) were formed in similar positions in all specimens.

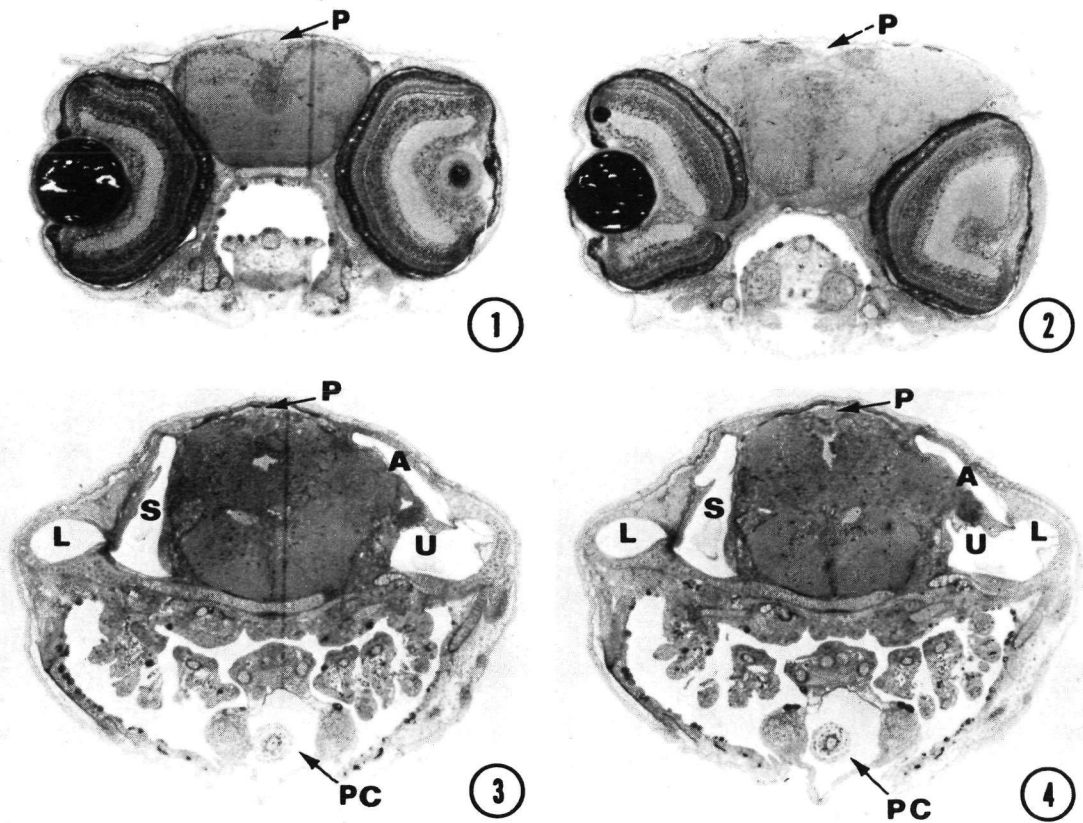
Further evidence of the normality of central nervous system development under spaceflight conditions is observed in the patterns of differentiation of both pineal and pituitary glands. The pineal consists of an elongated tubular profile with an enlarged terminal saccule (see P in figs. 1 thru 6 and PIN in fig 7). The pineal appears to be a secretory-type structure composed of columnar cells encompassing the central lumen and gives no evidence of any light sensitive structures in these young animals. The pituitary gland follows normal differentiation patterns with respect to cessation of labelling with ³H-thymidine and termination of ontogenetic cell death. Regions of continuing

cellular proliferation were observed in the midline ependyma as well as in marginal areas of hypo- and epithalamic regions (see zones labelled with P on figs. 7 and 8). Continuing ontogenetic cell death was a further indicator of the progressive differentiation in these regions (see arrows in figs. 7 and 8).

The neuropil of the central nervous system demonstrated normal vestibular projection profiles and central integrative structures. Such projection fibers could be followed due to their inherent staining densities in the preparations of serial sections utilized (see asterisks in fig 11). One of the more readily observable and quantitated CNS structures are the Mauthner cell bodies with their prominent dendrites and myelinated terminal axons (see M and arrows in figs. 6 and 12). In all specimens examined the Mauthner cells were similar (within sample limitations) in size, position and dendrite distribution. Based upon light microscopic surveys of surface densities, no differences were observed in the patterns of synaptic terminations upon the dendritic surface.

The eye develops normally with all retinal layers demonstrating continued proliferative activity only at the ora serrata. Ontogenetic cell death continues to outline the developing plexiform layers (see arrow in fig. 5). The optic nerve (ON), optic chiasm (OC) and optic tract (OT) developed in normal time and density in all specimens.

An abnormality of the vitreous humor space, previously observed in similar studies flown on the ASTP mission, is demonstrated in figure 5. The near total absence of the vitreous space was considerably more pronounced in both groups of spaceflown fish. It is our belief that this deficiency of vitreous space reflects an underlying fluid/electrolyte problem which is also involved in the production of the pericardial edema obvious in the spaceflown fish (see PC in figs. 3 and 4). Although edematous alterations in the vascular channels supplying the vestibular apparatus have been observed in all spaceflown specimens, no alterations to endolymphatic fluids, the responsible secretory epithelia or their basement membranes (see asterisk on figs. 9 and 10) have been observed.



Figures 1-4:

Selected serial section derived from K-1 - 32hour specimen at 22 days postfertilization. A, anterior semicircular canal; L, lateral semicircular canal; P, pineal gland; PC, pericardial sac; S, sacculus; U, utriculus. All figures 100x magnification.

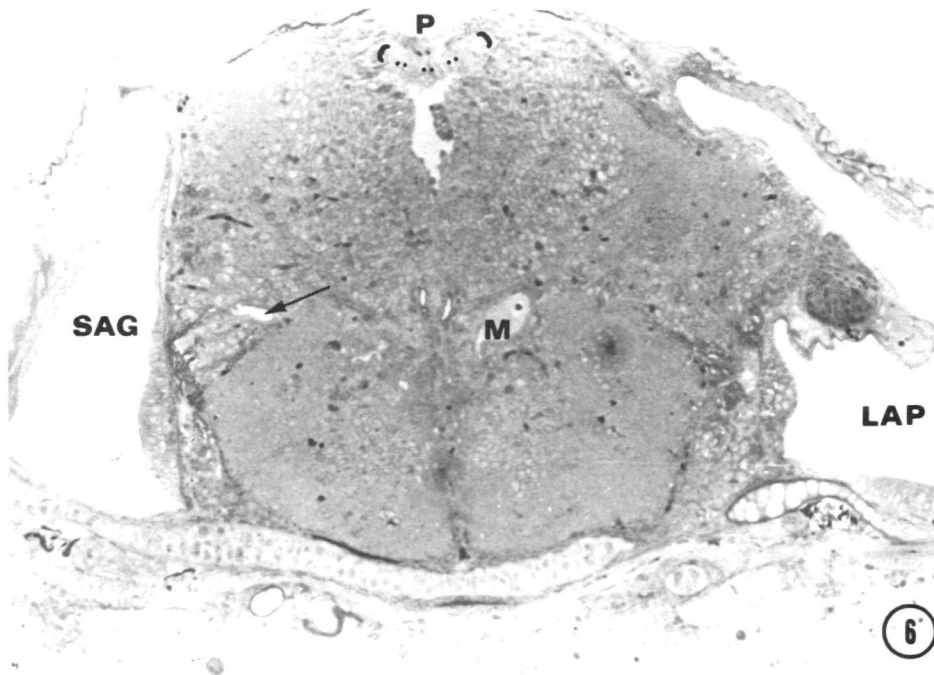
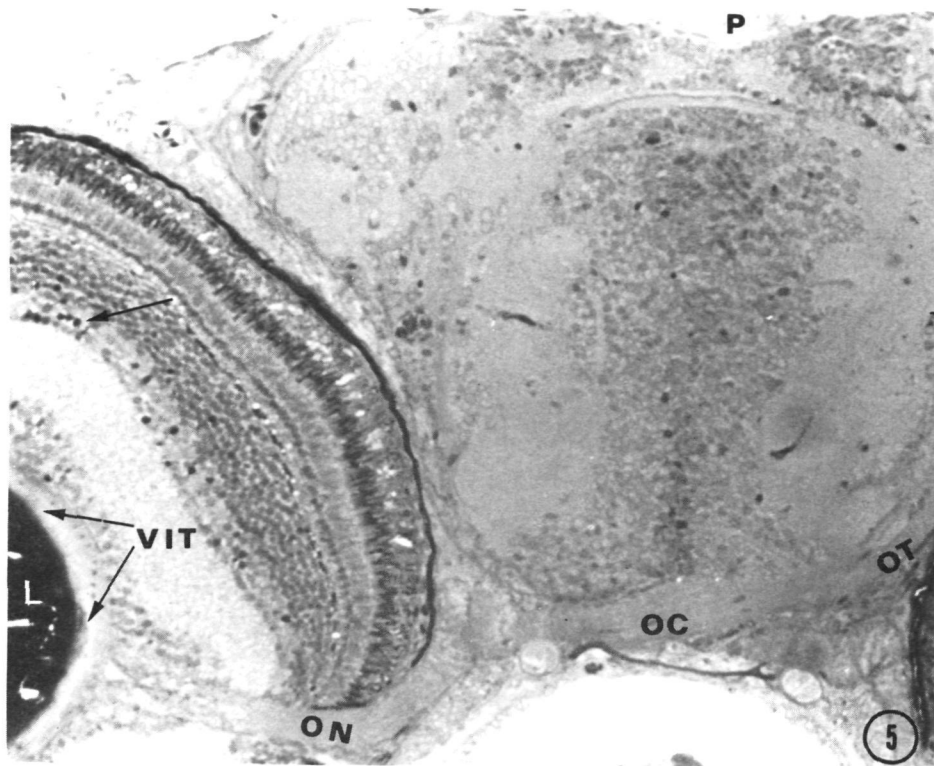


Figure 5: Detail through optic chiasm derived from Figure 2. ARROW, ontogenetic cell death on plexiform border; L, lens; OC, optic chiasm; ON, optic nerve; OT, optic tract; P, pineal gland; VIT, vitreous humor space. Magnification - 500x.

FIGURE 6: Detail of figure 4. LAP, lapillus (utricle statolith); M, Mauthner cell body (ARROW, Mauthner cell dendrite); P, pineal gland; (.. . . .) epithalamic/parapineal primordium; SAG, sagitta (Saccular statolith). Magnification - 300x.

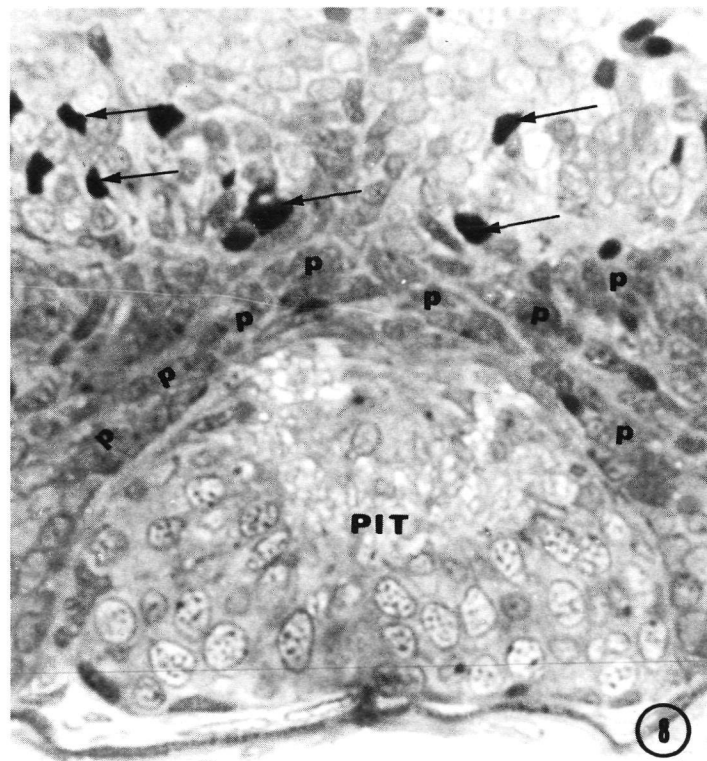
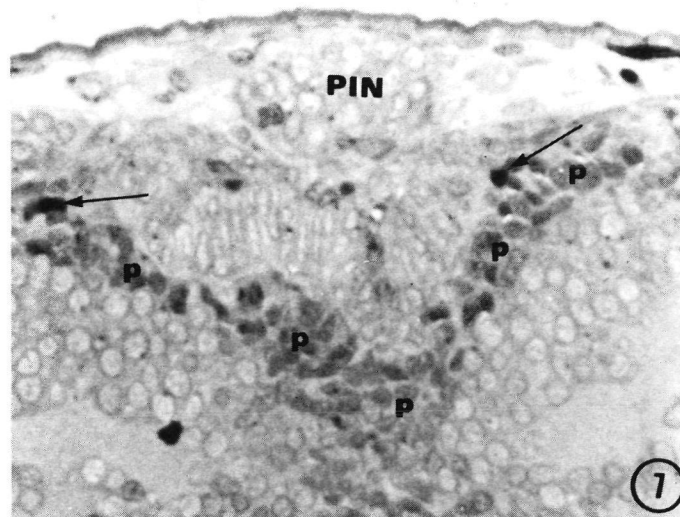


Figure 7: Detail through portion of tubular pineal gland of 32-hour flight animal. P, proliferative cell population in epithalamic region; PIN, tubular pineal gland; ARROWS, ontogenetic cell death. Magnification - 500x.

Figure 8: Detail of hypothalamic-hypophyseal region from a 32-hour flight animal. P, proliferative population in hypothalamic region; PIT, pituitary gland; ARROWS, ontogenetic cell death in differentiating zone. Magnification - 500x.

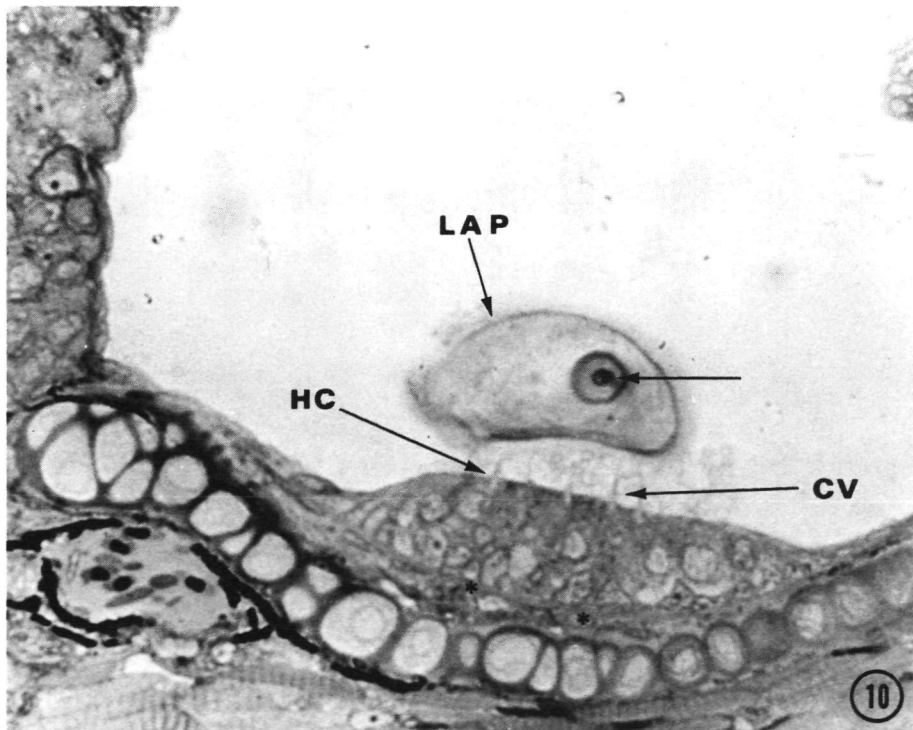
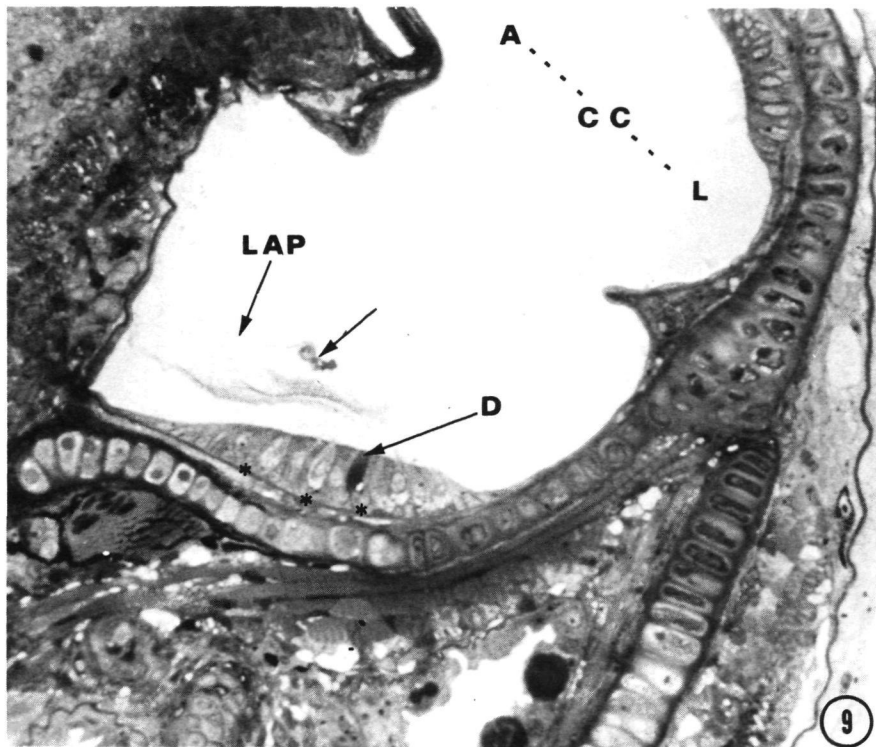


Figure 9: Detail of section through utricle of 32-hour flight animal. A---CC---L, anterior and lateral semicircular canals originating from common crus; ARROW, metachromatic dense granules in statolith; D, dark cell in macula; LAP, lapillus (utricular statolith); ASTERISKS, basement membrane of sensory macula. Magnification - 500x.

Figure 10: Detail of section through utricle of 44-hour flight animal (one gravity centrifuge specimen). ARROW, metachromatic dense granule of statolith; ASTERISKS, basement membrane of sensory macula; CV, cytoplasmic vacuole forming otolithic membrane; HC, hair cell of sensory macula; LAP, lapillus (utriculae statolith). Magnification - 1000x. 195

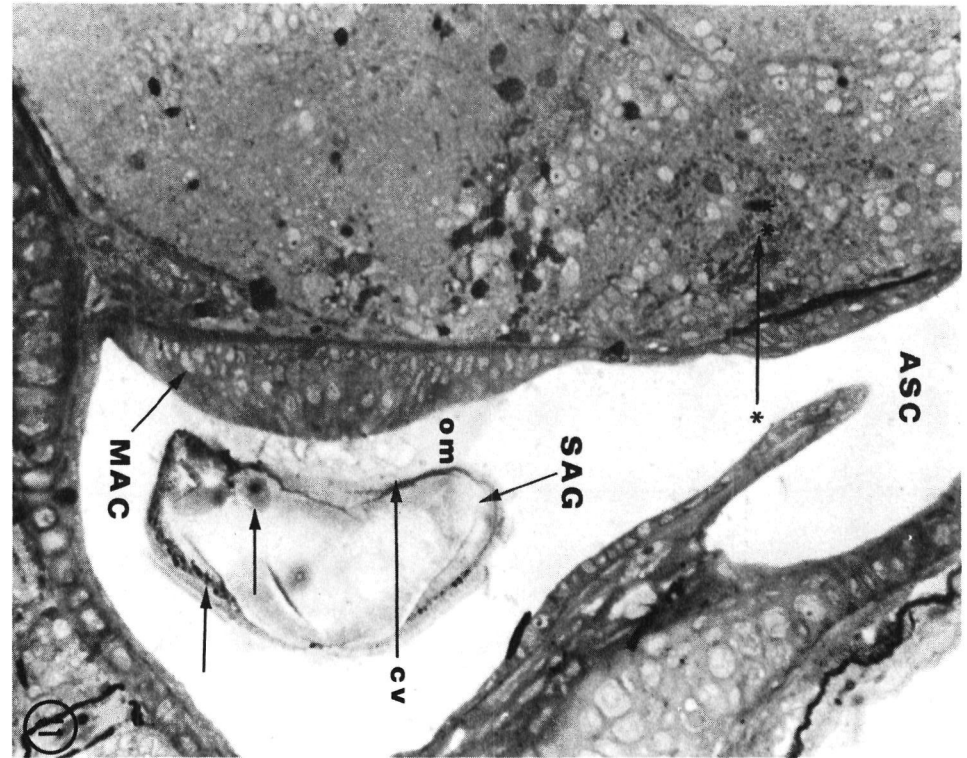
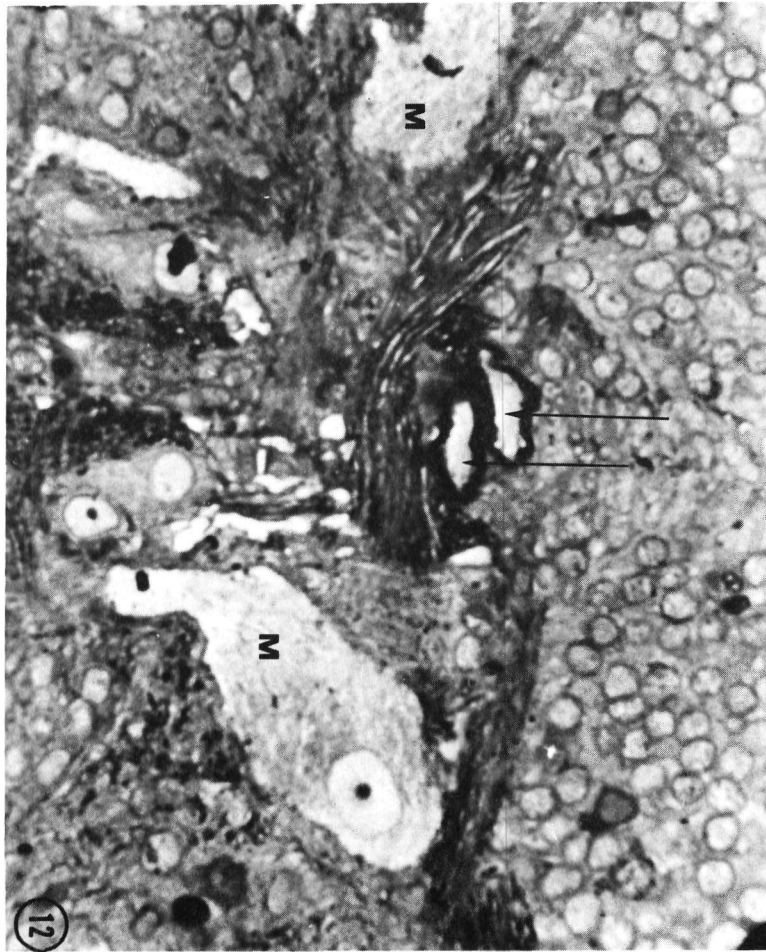


Figure 11: Detail of section through sacculus of 44-hour flight animal (one gravity centrifuge specimen). ARROWS, metachromatic dense granule and accretion line of statolith; ASC, anterior semicircular canal; ASTERISK, cross section of vestibular nerve root; CV, cytoplasmic vacuole forming otolith membrane; MAC, sensory macula of sacculus; OM, otolith membrane; SAG, sagitta (saccular statolith). Magnification - 1000 x.

Figure 12: Detail through central neuropil surrounding Mauthner cells from a 44-hour flight animal (one gravity centrifuge specimen). M, Mauthner cell body; ARROWS, Mauthner cell axons. Magnification-1100x.

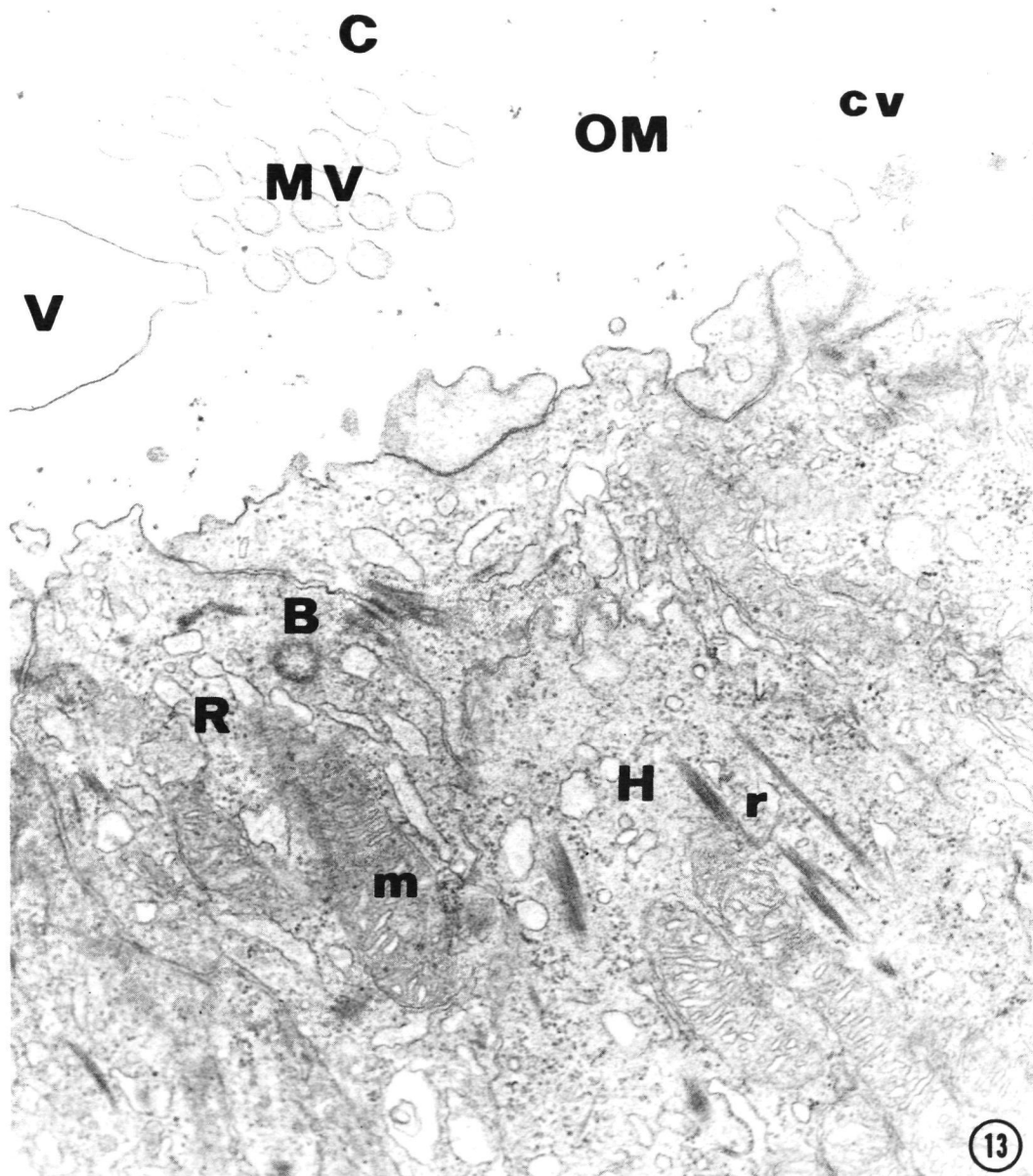


Figure 13: Electron micrograph of apical portion of sensory macula from the Utricle of a 128-hour flight animal. B, basal body; C, sensory kinocilium; CV, cytoplasmic vacuole forming otolithic membrane; H, hair cell of sensory macula; M, mitochondrion of sustentacular cell; MV, microvilli of hair cell surrounding kinocilium; OM, otolithic membrane between apical surface of sensory macula and statolith (not shown); R, distended cisternae of rough endoplasmic reticulum in sustentacular cell; r, ciliary rootlets of hair cell; V, apparently empty cytoplasmic vacuole. Magnification - 20,000x.

Cause of Anomalous Development

Postflight testing of procedures and materials from the experiment indicates that the probable cause of the high incidence of anomalous development lies in the tape used to label the plastic bags. Tests have shown high anomalies even in unlabelled bags immersed in water containing labelled bags. Analysis of organic volatiles from the tape by gas chromatography/mass spectrometry has resolved only one major component, carbon disulfide, that has known toxic properties in the concentration range potentially available in the experimental packages. All other detectable volatile components were present in much lower concentration and none have known toxic properties. Tests with carbon disulfide have demonstrated the capability for causing death or delay and anomalous development in Fundulus embryos.

CONCLUDING REMARKS

The experiment series to which the present experiment belongs was begun to explore the possible deleterious effects of exposure to weightlessness upon development and growth of biological systems. The results obtained thus far from these experiments are very encouraging in that we can say with considerable confidence that development of Fundulus beyond the gastrula stage is not affected in any major way by weightlessness. At this point it seems somewhat doubtful that we will discover very many minor effects, and it is tempting to speculate that weightlessness may be largely beneficial.

Most discussions of effects of weightlessness upon development appear to assume the weightless state to be a stressful condition. Practical experience with Fundulus development would suggest the opposite. There was indication of generally better health in flight animals of all three experiments of this series. With the possible exceptions of those aspects of development in which gravity is required as a cue for establishment of polarity or as a reference stimulus for sensory development, it is probable that the weightless state provides generally superior conditions for embryo development. This may not be true for situations in which maternal-fetal interaction is part of the process, but even there, the source of stress would be through the parents interaction with the environment while stress on the embryo and stress on the parent resulting directly from pregnancy should be reduced. Within the limits already noted, (i.e., establishment of polarity) there would seem to be no reason why earlier development of Fundulus from fertilization through gastrula should not also be enhanced. It is recommended that future experiments with the Fundulus be designed with this aspect as a prime consideration.

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ABSENCE OF GASTRIC ULCERATION IN RATS AFTER FLIGHT ON THE COSMOS 782

BIOLOGICAL SATELLITE

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ABSTRACT

Evidence of gastric ulceration or severe erosion of the gastric mucosa was sought in rats following 19.5 days of spaceflight on the Cosmos 782 Biological Satellite. The stomachs from the flight animals were compared macroscopically and histologically with stomachs removed from animals in the synchronous and vivarium control groups. None of the animals in the flight or the control groups ulcerated, and there were no obvious histologic differences in gastric erosion among the groups. The reasons for this failure to ulcerate are discussed.

INTRODUCTION

A variety of environmental stressors can be shown to produce gastric ulceration in rats (1,2,5,12,13). Several converging lines of research have shown that endogenous histamine plays an essential role in stress-produced gastric ulceration (6,9), and recently Brown et al. (4) demonstrated that the mechanism specifically involves the H₂ receptor for histamine. Pfeiffer (8) has reviewed evidence that certain of the stresses associated with spaceflight can produce changes in histamine levels in the gastrointestinal mucosa. Whether the actual stresses associated with modern long-term spaceflight are sufficient to produce gastric ulceration in rats is uncertain. The purpose of the present study was to determine the extent, if any, of gastric ulceration in rats subjected to 19.5 days of spaceflight aboard the Cosmos 782 Biosatellite.

METHODS

Male Wistar-line rats weighing between 250 and 300 g were divided into flight, synchronous control and vivarium control groups (Table 1). For the ulceration study, the stomachs were removed from 6 animals in each of the three groups. All 6 animals from the flight group received injections of C-14 glycine, listeria and declomycin. Five of the animals taken from the synchronous control group received injections of listeria and declomycin. None of the rats from the vivarium control group received any of the drugs. The administration of these drugs was required for other experiments which were concurrently performed on these animals.

A special diet developed for weightless conditions was delivered four times a day at 6-hr intervals to animals in all groups. Water was avail-

TABLE 1

EXPERIMENTAL CONDITIONS AND EXTENT OF GASTRIC ULCERATION

Group	N	Number of ulcers		
		Petechial	Punctate	Longitudinal
Flight ¹	6	0	0	0
Synchronous ²	6	0	0	0
Vivarium ³	6	0	0	0

¹All rats injected with C-14 glycine, declomycin and listeria

²Five rats injected with declomysin and listeria

³None of the rats received any of the drugs

able ad libitum. Details of the environmental conditions and hardware used to house the animals are described elsewhere (14).

The stomachs were removed from the flight animals within 5 to 11 hr after landing of the biosatellite and they were removed from the synchronous and vivarium control groups five to six days later. An incision was made on the ventral surface of each rat and the stomach was removed, cut along the greater curvature, rinsed with tap water to remove any contents, and placed in a vial containing buffered neutral formalin. The stomachs were examined macroscopically for gastric ulceration using the following classification (7): (a) Petechial ulcers: minute superficial erosions involving only the mucosa; (b) Punctate ulcers: discrete punched out ulceration measuring at least 1 mm; and (c) Longitudinal ulcers: continuous linear ulceration larger than 1 mm.

Tissue for histological examination was taken from the rumen, corpus and antrum from animals in each of the groups, embedded in paraffin and sectioned at 8 μ . Hematoxylin and eosin stained sections were examined microscopically for evidence of superficial erosion of the mucosa.

RESULTS

The results of the macroscopic examination of the gastric mucosa were unequivocal. None of the animals examined from the three experimental groups evidenced gastric ulcers or pronounced mucosal erosion (Table 1). It should be pointed out that macroscopic detection of very small petechial ulcers in formalin-fixed tissue is difficult. However the failure to find petechial ulcers was supported by microscopic examination of the H & E material, which also revealed no significant differences in gastric erosion in animals from the three groups.

DISCUSSION

The present investigation indicates that the rats subjected to prolonged spaceflight on the Cosmos 782 Biosatellite failed to evidence gastric ulceration or pronounced mucosal erosion upon return. For several reasons, this failure to ulcerate was not unexpected. First, all the animals were regularly fed four times a day at 6-hr intervals. In order to produce experimental stress ulcers in rats, a minimum of 24-hr of food deprivation is usually required (3). Second, in conjunction with other experiments, most of the animals in the flight and synchronous control groups were maintained on the antibiotic, declomycin (Table 1). Certain antibiotics have been shown to prevent or reduce gastric ulceration in rats (7,11). It has been found (7) that reductions in the bacterial flora of the gut with broad spectrum antibiotics can result in a deficiency of pyridoxal phosphate, which is a required co-factor in the biosynthesis of histamine. Other evidence has demonstrated that histamine depletion can afford protection against experimental ulcers in rats (10,15). Third, it is known that recovery from stress ulcers in rats is essentially complete within a few days (1); and unlike gastric ulceration in man, there is no residual scarring in rats. It is therefore possible that during the initial phases of the spaceflight, the animals did ulcerate, but recovered prior to the return of the biosatellite. The preceding considerations are probably sufficient to account for the failure of the rats to ulcerate. However it should also be kept in mind that the conditions of spaceflight on Cosmos 782 may have been considerably less stressful than the experimental procedures typically devised (2,4,13) to produce stress ulcers in rats.

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EFFECT OF SPACE FLIGHT ON CELL-MEDIATED IMMUNITY

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ABSTRACT

The cell mediated immune response to Listeria monocytogens was studied in rats subjected to 19.5 days of flight in a Soviet spacecraft. Groups of rats were immunized with 10^6 formalin killed Listeria suspended in Freund's Complete adjuvant five days prior to flight. Immunized rats subjected to the same environmental parameters as the flight rats, excepting flight, and immunized and non-immunized rats held in a normal animal colony served as controls. Following recovery, lymphocyte cultures were prepared from spleens of all rats, and cultured in vitro in the presence of Listeria antigens, phytohemagglutinin, Concanavalin A and purified protein derivative (PPD) and measured for their uptake of ^3H -thymidine. The lymphocytes of all rats gave a blastogenic response to phytohemagglutinin and Concanavalin A. Although individual rats varied considerably, all flight and immunized control rats gave a blastogenic response to the Listeria antigens and PPD. With several mitogens the lymphocytes of flight rats showed a significantly increased response over the controls. The data do not support a hypothesis of a detrimental effect of space flight on cell mediated immunity and suggest an opposite effect.

INTRODUCTION

Immunity to pathogenic Mycobacteria (1,3,11,12), Brucella (3,4,9), Listeria (2,5), Salmonella (7,8,10) and Candida (6) is not dependent on the production of high titers of circulating antibody produced by vaccines or the passive transfer of immune sera. Protection against these organisms or immunity to infection against them depends upon the presence in the host of mononuclear cells, the most important of which are lymphocytes and mononuclear phagocytes with increased antibacterial activity. Thus, cell mediated immunity has been shown to be responsible for certain types of responses to a variety of microbial and viral antigens, transplantation, autogenous and tumor specific antigens. It has been demonstrated that cell-mediated immune reactions in mammals are thymus dependent and that thymus derived, or thymus dependent lymphocytes (T-cells) are responsible for the reactions of cellular immunity. The T cells or T lymphocytes are derived from stem cells in the bone marrow, whose progeny migrate to the thymus where they acquire the capacity to perform certain immunological functions. T cells are also mobilized in the spleen where it has been shown that spleen cells from Listeria infected mice can transfer immunity to normal non-immune recipients. Rats flown in the Cosmos 605 space flights have shown changes in the size of thymus and spleen compared to ground-based controls. In addition, investigations on the second day after the end of the space flight showed that prolonged space flight caused hypoplasia of the red and white pulp of the spleen and hypoplasia of the thymus. These observations from the Cosmos 605 flight suggested a consideration of the effect that space flight might have on the cellular aspect of immune responsiveness. This study examined this effect of space flight on cell mediated immunity by examining rats infected with a formalin killed culture of Listeria monocytogenes suspended in Complete

Freunds adjuvant prior to flight. The uptake of ^3H -thymidine by the lymphocytes of immunized flight rats in the presence of specific antigen, was compared with those of immunized ground control rats.

MATERIALS AND METHODS

Vaccine Preparation

A culture of Listeria monocytogenes, obtained from Dr. A. Blazkovec of the Department of Medical Microbiology, University of Wisconsin, Madison, was used in these studies. Formalin killed L. monocytogenes in Complete Freunds adjuvant was used to immunize rats and stimulate a cell mediated immune response. L. monocytogenes was grown in a liter of brain heart infusion broth (Difco Laboratories Detroit, Michigan) for 18 hr at 37°C. The number of viable bacteria per ml was determined by plating (0.1 ml) serial dilutions for the 18 hr culture on brain heart infusion agar (Difco Laboratories, Detroit, Michigan). The bacterial culture was centrifuged at 10,000 xg for 10 min, the supernatant discarded and 3 vol of 0.5% formalin was added per ml of packed cells. After mixing and incubation at 37°C for 24 hr the supernatant was removed by centrifugation at 10,000 xg for 10 min and the formalinized cells were washed (by alternate centrifugation and resuspension) 3 times with sterile 0.85% sodium chloride. The washed bacteria were finally suspended in 10 cc of 0.85% sodium chloride. Sterility of the formalinized cell suspension was assessed by inoculating 0.1 ml of the final saline suspension of cells into brain heart infusion broth and incubating at 37°C for 24 hr. The formalin killed bacteria were added to Freunds Complete adjuvant (Difco Laboratories, Detroit, Michigan) with constant agitation until a ratio of 1:1

(vol:vol) was reached. The final concentration of dead cells was 10^7 /ml. The vaccine was stored in sterile rubber stoppered vaccine bottles (10cc) at 4°C until used. Rats were injected 5 days prior to the flight and the assays were performed on splenic lymphocytes from rats that were immunized 26 days previously.

Lymphocyte Preparation

Lymphocytes were obtained aseptically from the spleen of decapitated rats. After aseptic removal, the spleens were placed in RPMI-1640 medium (Gibco, Grand Island, New York) and transported (30 hr from landing site to laboratory) to the laboratory at 4°C. The excised spleens were each placed separately on a sterile 1" x 1" piece of stainless steel screen (#60 mesh) in a petri dish and washed with 8 ml of pH 7.0 phosphate buffered saline. The blunt end of a glass plunger (from a 5 cc glass syringe) was used to macerate the spleen until only a thin pulp of the spleen remained. The screen and petri dish were washed with 3 ml of phosphate buffered saline and, with a pasteur pipette, the fluid was placed in a centrifuge tube and allowed to stand for 5 min to permit the large clumps of spleen tissue to settle out. The lymphocyte rich supernatant fluid was then decanted into another centrifuge tube and centrifuged at 180 xg for 10 min. The supernatant was discarded and the packed cells were suspended in 1 ml of phosphate buffered saline. To disrupt red blood cells, 9 ml of sterile distilled water was added to the cell suspension and mixed with a pipette for 5 sec. Then, 1 ml of 10 X phosphate buffered saline was added to restore isotonicity. Some debris, which is usually present at this time, settles to the bottom and was removed by pipette. The cells were washed once with 5 ml phosphate buffered saline and then resuspended in RPMI-1640 medium. The concentration of viable lymphocytes was determined by diluting 50 μ l of cell suspension in 200 μ l of 0.5%

trypan Blue (in 0.8% saline) and counting in a hemocytometer. The viable lymphocyte concentration was then adjusted (with RPMI-1640) to a concentration of 1×10^6 viable lymphocytes/ml in RPMI-1640 medium containing 10% fresh, heated (60°C for 30 min) rat serum.

Mitogens

The contents of a vial of Phytohemagglutinin (PHA) and Concanavlin A (Con A) (Difco Laboratories, Detroit, Michigan) were each dissolved in 5 ml of distilled water and then diluted 1:10 and 1:100 with RPMI-1640 medium. Listeria whole cell antigen (WC), used in the blastogenesis assay, was prepared from a formalin killed culture of L. monocytogenes. A suspension of the latter cells was centrifuged at 10,000 xg for 10 min and suspended in RPMI-1640 medium to give a concentration of 1×10^7 cells/ml and then diluted 1:10 and 1:100 with RPMI-1640 medium. Antigen of the supernatant (cytoplasm) of L. monocytogenes was prepared by growing the culture in 250 ml of brain heart infusion broth at 37°C for 24 hr, centrifuging at 10,000 xg for 10 min, discarding the supernatant and washing the cells twice with 0.85% sodium chloride. Five ml of the latter cell suspension was passed through a French-Pressure Cell (Aminco Inst. Co., Silver Springs, Md.) and the fluid centrifuged at 10,000 xg for 30 min to remove all cellular debris. The supernatant fluid (sup) was adjusted to make concentrations of antigen at 100 µg and 50 µg protein/ml RPMI-1640 medium. Purified Protein Derivative (PPD) (generously supplied by NIH, Bethesda, Md.) was diluted 1:10 and 1:100 with RPMI-1640 medium and used in the assay for blastogenesis.

Lymphocyte Stimulation and ^3H -Thymidine Incorporation

The lymphocytes were dispensed in 0.1 ml aliquots in the microtest II plates (Falcon, Oxnard, Calif.). Aliquots of each lymphocyte culture were dispensed

in the microtest plates to permit a quadruplicate determination of the lymphocyte response to each mitogen. Two concentrations of each mitogen were tested in the assay and were added to the wells in 0.1 ml aliquots. Lymphocytes incubated in RPMI-1640 medium containing no mitogen served as background controls. A second set of controls consisted of mitogen alone in the presence of RPMI-1640 medium. The micro test plates were incubated at 37°C in an atmosphere of 5% CO₂ for 72 hr before adding 2 µCi of ³H-thymidine, in a volume of 0.05 ml of RPMI-1640 medium, to each well. The plates were incubated, as above, for 24 hr and finally precipitated on filter paper using the MASH II cell precipitator (Microbiological Associates, Baltimore, Md.). The precipitated cells were washed out of the wells and onto the filters (30X) with 0.9% sodium chloride. The filter strips were dried for 24 hr and individual filter discs were placed in scintillation vials containing 3 ml of liquiflour counting cocktail (New England Nuclear Corp., Boston, Mass.). Each sample was counted for 10 min in a Packard Tri-Carb liquid scintillation spectrometer, model 3370 (Packard Instr. Co., Downers Grove, Ill.). The results are expressed as the mean counts/ min/10⁵ lymphocytes and standard error of the mean (S.E.).

Calculation of Stimulation Index (SI)

The levels of mitogen induced lymphocyte blastogenesis were also calculated by comparing the mean counts/min of four replicate wells containing lymphocytes cultured in the presence of a given concentration of mitogen with the mean counts/min of four other replicate wells containing lymphocytes cultured in the absence of mitogen (controls).

Statistics

Student's T test was used to assess the significance of the data.

RESULTS

Table 1 shows the blastogenic response of splenic lymphocytes from individual flight animals after incubation with none or with two different concentrations of each specific and non-specific mitogen. The data is tabulated to show the mean, standard deviation and standard error of the mean of the counts per minute of ^3H -thymidine incorporated by quadruplicate samples of splenic lymphocytes ($10^5/\text{well}$) after stimulation with the specific and non-specific mitogens. The lymphocytes of flight rats responded well to the non-specific mitogens and to the bacterial antigens they were immunized with 26 days previously. Maximum thymidine incorporation occurred with lymphocytes exposed to the non-specific mitogens (Con A and PHA). The blastogenic response to the bacterial antigens was better with *Listeria* whole cells and PPD than with *Listeria* cytoplasmic antigen. Some of the best blastogenic activity occurred with lymphocytes from the flight rats (compare counts in table 1 with counts in tables 2, 3 and 4).

Table 2 shows the blastogenic response of the synchronous control rats. In general, the latter group did not show as good a blastogenic response as the ground based vivarium controls (table 3) or the flight rats (table 1). In fact, the synchronous control group (table 2), although they did respond to the specific and non-specific mitogens, had some of the poorest responding lymphocytes in the study. The vivarium control rats (table 3) showed generally good responses to the specific and non-specific mitogens. Of interest with the vivarium control group (table 3) was the fact that they had an excellent blastogenic response to the *Listeria* supernatant antigen. The flight and synchronous controls, however, did not respond very well to the *Listeria* supernatant antigen. The non-injected control rats (table 4) manifested little blastogenic response to the *Listeria* and PPD antigens; however,

Table 1. Effect of space flight on blastogenic response of rat splenic lymphocytes

FLIGHT RATS

Mitogens	1	2	3	4	5	6	Mean $\pm$ SD (SE)
A. 1-3 PHA ₂ + RPMI + 3H	54 $\pm$ 1.0	38 $\pm$ 4	35 $\pm$ 2	49 $\pm$ 6	55 $\pm$ 20	54 $\pm$ 9	47.7 $\pm$ 8.5 (3.5)
4-6 PHA ₂ + cells + 3H	161 $\pm$ 18	665 $\pm$ 99	162 $\pm$ 17	2290 $\pm$ 187	3655 $\pm$ 67	680 $\pm$ 20	1268.0 $\pm$ 1407.1 (574.5)
7-9 PHA ₁ + cells + 3H	190 $\pm$ 49	1775 $\pm$ 330	1455 $\pm$ 77	6640 $\pm$ 292	10683 $\pm$ 256	960 $\pm$ 200	3617.2 $\pm$ 4145.8 (1692.5)
10-12 RPMI + cells + 3H	42 $\pm$ 12	215 $\pm$ 133	127 $\pm$ 13	387 $\pm$ 60	226 $\pm$ 54	114 $\pm$ 17	185.2 $\pm$ 120.2 (49.1)
B. 1-3 ConA ₂ + RPMI + 3H	82 $\pm$ 8.4	82 $\pm$ 37	65 $\pm$ 16	61 $\pm$ 7	35 $\pm$ 7	52 $\pm$ 8	62.8 $\pm$ 18.1 (7.4)
4-6 ConA ₂ + cells + 3H	163 $\pm$ 96	218 $\pm$ 55	150 $\pm$ 25	636 $\pm$ 101	693 $\pm$ 181	1530 $\pm$ 240	565 $\pm$ 530.4 (216.6)
7-9 ConA ₁ + cells + 3H	1229 $\pm$ 175	4563 $\pm$ 578	1110 $\pm$ 160	3235 $\pm$ 387	2464 $\pm$ 87	1530 $\pm$ 620	2355.2 $\pm$ 1353.5 (552.6)
10-12 RPMI + cells + 3H	57 $\pm$ 8	187 $\pm$ 37	127 $\pm$ 13	350 $\pm$ 32	256 $\pm$ 54	125 $\pm$ 18	183.7 $\pm$ 105.5 (43.1)
C. 1-3 List.wc ₂ + RPMI + 3H	74 $\pm$ 26	69 $\pm$ 15	74 $\pm$ 40	37 $\pm$ 3	89 $\pm$ 28	65 $\pm$ 10	68 $\pm$ 17.2 (7.0)
4-6 List.wc ₂ + cells + 3H	301 $\pm$ 120	1053 $\pm$ 304	2000 $\pm$ 370	1814 $\pm$ 359	1965 $\pm$ 41	283 $\pm$ 52	1236 $\pm$ 808.1 (329.9)
7-9 List.wc ₁ + cells + 3H	240 $\pm$ 68	1302 $\pm$ 344	1204 $\pm$ 300	2067 $\pm$ 567	590 $\pm$ 41	950 $\pm$ 30	1058.8 $\pm$ 632.3 (258.1)
10-12 RPMI + cells + 3H	45 $\pm$ 3	44 $\pm$ 3	85 $\pm$ 9	287 $\pm$ 35	218 $\pm$ 43	88 $\pm$ 9	127.8 $\pm$ 100.8 (41.1)
D. 1-3 List.sup ₂ + RPMI + 3H	93 $\pm$ 3	49 $\pm$ 19	63 $\pm$ 8	56 $\pm$ 5	29 $\pm$ 6	15 $\pm$ 2.4	50.8 $\pm$ 27.3 (11.1)
4-6 List.sup ₂ + cells + 3H	107 $\pm$ 41	349 $\pm$ 64	278 $\pm$ 65	345 $\pm$ 40	679 $\pm$ 217	112 $\pm$ 29	311.7 $\pm$ 209.9 (85.7)
7-9 List.sup ₁ + cells + 3H	227 $\pm$ 132	835 $\pm$ 289	735 $\pm$ 200	110 $\pm$ 11	115 $\pm$ 8	435 $\pm$ 82	409.5 $\pm$ 315.4 (128.8)
10-12 RPMI + cells + 3H	56 $\pm$ 7	52 $\pm$ 8	99 $\pm$ 10	187 $\pm$ 15	187 $\pm$ 26	121 $\pm$ 15	117 $\pm$ 60.1 (24.6)
E. 1-3 PPD ₂ + RPMI + 3H	45 $\pm$ 4	47 $\pm$ 5	48 $\pm$ 10	42 $\pm$ 3	37 $\pm$ 3	63 $\pm$ 12	47 $\pm$ 8.8 (3.6)
4-6 PPD ₂ + cells + 3H	456 $\pm$ 123	672 $\pm$ 212	424 $\pm$ 38	1349 $\pm$ 90	3513 $\pm$ 262	1060 $\pm$ 280	1245.7 $\pm$ 1167.4 (476.6)
7-9 PPD ₁ + cells + 3H	410 $\pm$ 114	241 $\pm$ 26	261 $\pm$ 33	1927 $\pm$ 150	2921 $\pm$ 163	1240 $\pm$ 240	1166.7 $\pm$ 1087.3 (443.9)
10-12 RPMI + cells + 3H	55 $\pm$ 11	33 $\pm$ 3	45 $\pm$ 6	127 $\pm$ 6	115 $\pm$ 27	132 $\pm$ 27	84.5 $\pm$ 44.9 (18.3)

PHA₁ - Phytohemagglutinin 1:10PHA₂ - Phytohemagglutinin 1:100PPD₁ - Purified Protein Derivative of Mycobacterium Tuberculosis 1:10PPD₂ - Purified Protein Derivative of Mycobacterium Tuberculosis 1:100Con A₁ - Concanavlin 1:10Con A₂ - Concanavlin 1:100List wc₁ - Listeria whole cell antigen 1:10List wc₂ - Listeria whole cell antigen 1:100List sup₁ - Listeria supernatant 50 μ g proteinList sup₂ - Listeria supernatant 100 μ g protein

Table 2. Effect of simulated space flight on the blastogenic response of rat splenic lymphocytes.

Mitogens	Synchronous Controls						Synchronous Controls			
	7	8	9	10	11	12	x	±	SD	(SE)
A. 1-3 PHA ₂ + RPMI + 3 _H	191 ± 67	55 ± 14	85 ± 11	63 ± 15	29 ± 2	87 ± 22	85	±	56.1	(22.9)
4-6 PHA ₂ + cells + 3 _H	370 ± 64	91 ± 29	80 ± 5	99 ± 10	60 ± 10	1234 ± 396	322.3	±	461.4	(188.4)
7-9 PHA ₁ + cells + 3 _H	250 ± 115	140 ± 28	131 ± 41	117 ± 29	94 ± 14	277 ± 65	168.2	±	75.9	(31.0)
10-12 RPMI + cells + 3 _H	323 ± 135	65 ± 11	49 ± 0.8	44 ± 4	51 ± 4	235 ± 81	127.8	±	120.6	(49.2)
B. 1-3 ConA ₂ + RPMI + 3 _H	386 ± 96	183 ± 105	72 ± 7	69 ± 24	35 ± 4	378 ± 140	187.2	±	158.9	(64.9)
4-6 ConA ₂ + cells + 3 _H	468 ± 127	197 ± 61	70 ± 9	126 ± 12	130 ± 38	2291 ± 616	547	±	865.9	(353.5)
7-9 ConA ₁ + cells + 3 _H	237 ± 140	79 ± 12	137 ± 42	1188 ± 88	90 ± 15	858 ± 292	431.5	±	473.2	(193.2)
10-12 RPMI + cells + 3 _H	72 ± 8	72 ± 23	49 ± 3	61 ± 6	41 ± 3	554 ± 172	141.5	±	202.5	(82.7)
C. 1-3 List.wc ₂ + RPMI + 3 _H	84 ± 40	46 ± 4	55 ± 8	33 ± 5	31 ± 2	31 ± 2	46.7	±	20.7	(8.5)
4-6 List.wc ₂ + cells + 3 _H	261 ± 129	142 ± 53	165 ± 89	170 ± 28	276 ± 88	517 ± 45	255.2	±	139.4	(56.9)
7-9 List.wc ₁ + cells + 3 _H	271 ± 76	100 ± 16	169 ± 62	83 ± 11	129 ± 30	1000 ± 526	292	±	353.3	(144.2)
10-12 RPMI + cells + 3 _H	170 ± 47	58 ± 6	88 ± 17	53 ± 5	54 ± 10	122 ± 23	90.8	±	47.1	(19.3)
D. 1-3 List.sup ₂ + RPMI + 3 _H	151 ± 67	38 ± 3	56 ± 2	52 ± 5	196 ± 98	88 ± 27	96.8	±	63.2	(25.8)
4-6 List.sup ₂ + cells + 3 _H	99 ± 21	199 ± 46	179 ± 44	220 ± 79	317 ± 58	494 ± 171	251.3	±	138.1	(56.4)
7-9 List.sup ₁ + cells + 3 _H	163 ± 30	391 ± 135	191 ± 94	212 ± 34	216 ± 58	612 ± 140	297.5	±	173.8	(70.9)
10-12 RPMI + cells + 3 _H	133 ± 10	54 ± 5	41 ± 2	68 ± 5	44 ± 5	196 ± 81	86	±	59.9	(24.5)
E. 1-3 PPD ₂ + RPMI + 3 _H	56 ± 7	39 ± 1	80 ± 38	70 ± 16	66 ± 24	39 ± 3	58.3	±	16.8	(6.9)
4-6 PPD ₂ + cells + 3 _H	135 ± 39	92 ± 26	215 ± 109	156 ± 25	178 ± 47	78 ± 14	142.3	±	51.9	(21.2)
7-9 PPD ₁ + cells + 3 _H	139 ± 12	196 ± 63	268 ± 150	244 ± 24	158 ± 56	114 ± 18	186.5	±	60.6	(24.7)
10-12 RPMI + cells + 3 _H	53 ± 8	54 ± 5	51 ± 7	40 ± 4	193 ± 80	43 ± 3	72.3	±	59.4	(24.2)

Table 3. Blastogenic response of (vivarium) lymphocytes from rat spleens

Mitogens	Immunized Ground (vivarium) Controls					Ground Controls			
	13	14	15	16	17	$\bar{x}$	$\pm$	SD	(SE)
A. 1-3 PHA ₂ + RPM1 + 3 _H	49 $\pm$ 7	120 $\pm$ 4	34.7 $\pm$ 8.7	50 $\pm$ 8	41.7 $\pm$ 12	59.1	$\pm$	34.6	(15.5)
4-6 PHA ₂ + cells + 3 _H	154 $\pm$ 5	105 $\pm$ 76	443 $\pm$ 12.7	633 $\pm$ 12	497 $\pm$ 13.8	366.4	$\pm$	227.7	(101.8)
7-9 PHA ₁ + cells + 3 _H	161 $\pm$ 2	100 $\pm$ 22	443 $\pm$ 13.3	680 $\pm$ 23	254 $\pm$ 52	327.6	$\pm$	235.8	(105.5)
10-12 RPM1 + cells + 3 _H	37 $\pm$ 5	36 $\pm$ 4.6	30 $\pm$ 16.8	41 $\pm$ 12	39 $\pm$ 8	36.6	$\pm$	4.2	(1.9)
B. 1-3 ConA ₂ + RPM1 + 3 _H	76 $\pm$ 9	461 $\pm$ 6.7	347 $\pm$ 27	53 $\pm$ 5.3	350 $\pm$ 12	257.4	$\pm$	182.2	(81.5)
4-6 ConA ₂ + cells + 3 _H	150 $\pm$ 16	554 $\pm$ 93	417 $\pm$ 10.7	623 $\pm$ 9.2	480 $\pm$ 11	444.8	$\pm$	182.1	(81.4)
7-9 ConA ₁ + cells + 3 _H	160 $\pm$ 15	87 $\pm$ 37	266 $\pm$ 17.6	761 $\pm$ 167	1409 $\pm$ 184	536.6	$\pm$	554.2	(247.9)
10-12 RPM1 + cells + 3 _H	49 $\pm$ 5	67 $\pm$ 17	30.3 $\pm$ 8.1	40.7 $\pm$ 12	34.3 $\pm$ 6	44.06	$\pm$	14.7	(6.6)
C. 1-3 List.wc ₂ + RPM1 + 3 _H	90 $\pm$ 9	562 $\pm$ 2.6	48 $\pm$ 41	69 $\pm$ 21	780 $\pm$ 19	329.8	$\pm$	322.2	(144.1)
4-6 List.wc ₂ + cells + 3 _H	266 $\pm$ 25	494 $\pm$ 33	63 $\pm$ 10.3	590 $\pm$ 81	824 $\pm$ 24	447.4	$\pm$	293.7	(131.3)
7-9 List.wc ₁ + cells + 3 _H	345 $\pm$ 25	473 $\pm$ 43	370 $\pm$ 9.3	547 $\pm$ 63	827 $\pm$ 19	512.4	$\pm$	193.7	(86.6)
10-12 RPM1 + cells + 3 _H	41 $\pm$ 4	76 $\pm$ 15	37 $\pm$ 9.3	53 $\pm$ 10	39.3 $\pm$ 9.9	50.46	$\pm$	15.6	(7)
D. 1-3 List.sup ₂ + RPM1 + 3 _H	47 $\pm$ 9	66 $\pm$ 3.8	59 $\pm$ 4.7	96 $\pm$ 36	71 $\pm$ 28	67.8	$\pm$	18.2	(8.1)
4-6 List.sup ₂ + cells + 3 _H	36 $\pm$ 30	419 $\pm$ 25	530 $\pm$ 6.5	583 $\pm$ 28	813 $\pm$ 4.8	541.2	$\pm$	175.5	(78.5)
7-9 List.sup ₁ + cells + 3 _H	460 $\pm$ 43	497 $\pm$ 23	410 $\pm$ 7	503 $\pm$ 13	530 $\pm$ 16	480	$\pm$	46.4	(20.8)
10-12 RPM1 + cells + 3 _H	36 $\pm$ 2	32 $\pm$ 13	51 $\pm$ 24	49 $\pm$ 8.6	36 $\pm$ 8	40.8	$\pm$	8.6	(3.8)
E. 1-3 PPD ₂ + RPM1 + 3 _H	45 $\pm$ 2	33 $\pm$ 11.0	34 $\pm$ 5.3	43 $\pm$ 92	35 $\pm$ 8	38	$\pm$	5.6	(2.5)
4-6 PPD ₂ + cells + 3 _H	132 $\pm$ 2	367 $\pm$ 14.7	440 $\pm$ 15.9	493 $\pm$ 164	847 $\pm$ 50	456	$\pm$	258.2	(115.5)
7-9 PPD ₁ + cells + 3 _H	232 $\pm$ 2	400 $\pm$ 10.7	307 $\pm$ 6.0	460 $\pm$ 81	660 $\pm$ 58	411.8	$\pm$	163.8	(73.3)
10-12 RPM1 + cells + 3 _H	33 $\pm$ 2	35.7 $\pm$ 8	34.3 $\pm$ 10.7	35 $\pm$ 12	42 $\pm$ 12	36	$\pm$	3.5	(1.6)

Table 4. Blastogenic response of rat splenic lymphocytes

Nonimmunized Controls
Mean $\pm$ SD

Mitogens	18	19	20	X	$\pm$ SD	SE
A. 1-3 PHA ₂ + RPMI + 3 _H	29 $\pm$ 3.9	63 $\pm$ 14	46 $\pm$ 11	46	$\pm$ 17	(9.8)
4-6 PHA ₂ + cells + 3 _H	343 $\pm$ 440	643 $\pm$ 92	104 $\pm$ 200	363.3	$\pm$ 270	(155.9)
7-9 PHA ₁ + cells + 3 _H	480 $\pm$ 380	587 $\pm$ 430	165 $\pm$ 187	410.7	$\pm$ 219.4	(126.7)
10-12 RPMI + cells + 3 _H	32 $\pm$ 8	38 $\pm$ 4.8	40 $\pm$ 12	36.67	$\pm$ 4.2	(2.4)
B. 1-3 ConA ₂ + RPMI + 3 _H	33 $\pm$ 6.8	56 $\pm$ 7	48 $\pm$ 8	45.7	$\pm$ 11.7	(6.7)
4-6 ConA ₂ + cells + 3 _H	297 $\pm$ 160	493 $\pm$ 92	470 $\pm$ 69	420	$\pm$ 107.1	(61.8)
7-9 ConA ₁ + cells + 3 _H	673 $\pm$ 99	723 $\pm$ 89	369 $\pm$ 62	588.3	$\pm$ 191.6	(110.6)
10-12 RPMI + cells + 3 _H	37 $\pm$ 10	46 $\pm$ 11	41 $\pm$ 68	41.3	$\pm$ 4.51	(2.6)
C. 1-3 List.wc ₂ + RPMI + 3 _H	46 $\pm$ 7	35 $\pm$ 7	47 $\pm$ 8.5	66	$\pm$ 44.5	(25.7)
4-6 List.wc ₂ + cells + 3 _H	44 $\pm$ 9.4	44 $\pm$ 7	40 $\pm$ 25	42.7	$\pm$ 2.3	(1.3)
7-9 List.wc ₁ + cells + 3 _H	34 $\pm$ 8.4	45 $\pm$ 11	32 $\pm$ 14	37	$\pm$ 7	(4.4)
10-12 RPMI + cells + 3 _H	31 $\pm$ 3.4	34 $\pm$ 8	39 $\pm$ 7	34.7	$\pm$ 4.0	(2.3)
D. 1-3 List.sup ₂ + RPMI + 3 _H	59 $\pm$ 6.8	58 $\pm$ 13	57 $\pm$ 23	58	$\pm$ 1	(0.6)
4-6 List.sup ₂ + cells + 3 _H	45 $\pm$ 12	40 $\pm$ 6	80 $\pm$ 9.6	55	$\pm$ 21.8	(12.6)
7-9 List.sup ₁ + cells + 3 _H	40 $\pm$ 4	42 $\pm$ 11	59 $\pm$ 10	47	$\pm$ 10.4	(6.0)
10-12 RPMI + cells + 3 _H	36 $\pm$ 16	46 $\pm$ 9	43 $\pm$ 13.9	41.7	$\pm$ 5.1	(2.9)
E. 1-3 PPD ₂ + RPMI + 3 _H	46 $\pm$ 12	40 $\pm$ 10	34 $\pm$ 2.3	40	$\pm$ 6	(3.46)
4-6 PPD ₂ + cells + 3 _H	47 $\pm$ 9	39 $\pm$ 10	75 $\pm$ 9.8	53.7	$\pm$ 18.9	(10.9)
7-9 PPD ₁ + cells + 3 _H	44 $\pm$ 11	62 $\pm$ 15	81 $\pm$ 2.8	62.3	$\pm$ 18.5	(10.7)
10-12 RPMI + cells + 3 _H	38 $\pm$ 10	45 $\pm$ 16	50 $\pm$ 13	44.3	$\pm$ 6.0	(3.5)

their response to the non-specific mitogen (Con A and PHA) was comparable to the response of flight (table 1) and vivarium control (table 3) groups of rats.

Table 5 summarizes and presents the statistically significant differences observed. The ground based vivarium controls were compared with the flight and synchronous control groups using the counts per minute of ^3H -thymidine incorporated per 10^5 cells as a basis. Because of the large standard deviations that occurred, it was not possible to place much significance on the data. There were, however, several observations of importance. The overall level of incorporation by lymphocytes from flight rats were generally higher than the control groups for both specific and non-specific mitogens. The non-stimulated lymphocyte from flight rats showed higher background levels (significant at 5% level) in several instances when compared to levels observed in the ground controls. Of particular interest also in the heightened response of flight rat lymphocytes to *Listeria* supernatant antigen in comparison to ground controls. The response of Lymphocytes from synchronous controls (table 5) in comparison to ground based (vivarium) controls was also generally poor and the lack of response to *Listeria* supernatant and PPD are particularly noteworthy.

Blastogenesis data is also expressed as a stimulation index (see Materials and Methods). Using the calculated stimulation index (table 6) it is evident that the lymphocytes from synchronous control rats did not respond to *Listeria* supernatant, PPD or PHA as well as the ground controls. Also, lymphocytes from flight rats responded poorer than the vivarium controls to the *Listeria* supernatant antigen. As expected, the lymphocytes from non-injected controls did not respond to the bacterial antigens.

DISCUSSION

The blastogenic response of lymphocytes has not been studied as extensively in rats as it has been in mice and guinea pigs, and we do not know how represent-

Table 5. Summary of blastogenic response of
lymphocytes from flight and control rats
Mean $\pm$ S.D. (S.E.) of Counts
(cpm/10⁵ Lymphocytes)

Mitogens	Flight (6)	Synchronous (6)	Ground(vivarium) (5)	Nonimmunized (3)
A. 1-3 PHA ₂ + RPM1 + 3 _H	47.7 $\pm$ 8.5 (4)	85 $\pm$ 56 (23)	59 $\pm$ 34 (15)	46 $\pm$ 17 (10)
4-6 PHA ₂ + cells + 3 _H	1268.8 $\pm$ 1407 (575)	322 $\pm$ 461 (188)	366 $\pm$ 228 (102)	363 $\pm$ 270 (155)
7-9 PHA ₁ + cells + 3 _H	3617 $\pm$ 4145 (1693)	168 $\pm$ 75 (31)	327 $\pm$ 236 (105)	410 $\pm$ 219 (126)
10-12 RPM1 + cells + 3 _H	185.2 $\pm$ 120 (49.1)*	127 $\pm$ 120 (49)	37 $\pm$ 4 (2)	37 $\pm$ 4 (2)
B. 1-3 CONA ₂ + RPM1 + 3 _H	62.8 $\pm$ 18 (7)	187 $\pm$ 158 (65)	257 $\pm$ 182 (81)	46 $\pm$ 12 (7)
4-6 CONA ₂ + cells + 3 _H	565 $\pm$ 530 (217)	547 $\pm$ 866 (354)	448 $\pm$ 182 (81)	420 $\pm$ 107 (62)
7-9 CONA ₁ + cells + 3 _H	2355 $\pm$ 1353 (553)*	431 $\pm$ 473 (193)	536 $\pm$ 554 (248)	588 $\pm$ 191 (111)
10-12 RPM1 + cells + 3 _H	184 $\pm$ 105 (43)*	142 $\pm$ 202 (83)	44 $\pm$ 15 (7)	41 $\pm$ 4 (3)
C. 1-3 List wc ₂ + RPM1 + 3 _H	68 $\pm$ 17 (7)	47 $\pm$ 21 (8)	330 $\pm$ 322 (144)	66 $\pm$ 44 (26)
4-6 List wc ₂ + cells + 3 _H	1236 $\pm$ 808 (330)*	255 $\pm$ 139 (57)	447 $\pm$ 294 (131)	43 $\pm$ 2 (1)*
7-9 List wc ₁ + cells + 3 _H	1058 $\pm$ 633 (258)	292 $\pm$ 353 (144)	512 $\pm$ 193 (86)	37 $\pm$ 7 (4)*
10-12 RPM1 + cells + 3 _H	128 $\pm$ 100 (41)	91 $\pm$ 47 (19)	50 $\pm$ 16 (7)	35 $\pm$ 4 (2)
D. 1-3 List.sup ₂ + RPM1 + 3 _H	51 $\pm$ 27 (11)	97 $\pm$ 63 (26)	68 $\pm$ 18 (8)	58 $\pm$ 1 (1)
4-6 List.sup ₂ + cells + 3 _H	312 $\pm$ 210 (86)	251 $\pm$ 138 (56)*	541 $\pm$ 175 (78)	55 $\pm$ 22 (13)*
7-9 List.sup ₁ + cells + 3 _H	409 $\pm$ 315 (129)	298 $\pm$ 173 (71)*	480 $\pm$ 46 (20)	47 $\pm$ 10 (6)*
10-12 RPM1 + cells + 3 _H	117 $\pm$ 60 (25)*	86 $\pm$ 59 (24)	40 $\pm$ 9 (4)	42 $\pm$ 5 (3)
E. 1-3 PPD ₂ + RPM1 + 3 _H	47 $\pm$ 9 (4)	58 $\pm$ 17 (7)	38 $\pm$ 6 (2)	40 $\pm$ 6 (4)
4-6 PPD ₂ + cells + 3 _H	1245 $\pm$ 1167 (477)	142 $\pm$ 52 (21)*	456 $\pm$ 258 (115)	54 $\pm$ 19 (11)*
7-9 PPD ₁ + cells + 3 _H	1168 $\pm$ 1087 (444)	187 $\pm$ 61 (25)*	411 $\pm$ 163 (73)	62 $\pm$ 19 (11)*
10-12 RPM1 + cells + 3 _H	84 $\pm$ 45 (18)*	72 $\pm$ 59 (24)	36 $\pm$ 3 (2)	44 $\pm$ 6 (4)

* Statistically significant from ground (vivarium) controls at 5% level of significance.

Table 6. Effect of space flight and simulated space flight on the capacity of lymphocytes to respond to mitogens.

Mitogens	<u>Stimulation Indexes from Test and Control Rats</u>											
	Flight (6*)			Synchronous			Ground			Noninject.(3*)		
	(SD)	(SE)	(SI)	SD	(SE)	(SI)	SD	(SE)	(SI)	SD	(SE)	(SI)
PHA ₁	15.8	(6.4)	16.2	0.8	(0.3)	1.9**	6.3	(2.8)	9.0	6.4	(3.7)	11.5
PHA ₂	5.3	(2.1)	6.0	1.6	(0.65)	2.2**	5.9	(2.7)	10.0	7.2	(4.2)	10.1
CONA ₁	6.8	(2.8)	14.2	7.1	(2.9)	5.1	16.4	(7.3)	14.7	4.7	(2.7)	14.2
CONA ₂	4.2	(1.7)	3.7	1.8	(0.74)	3.3	5.1	(11.5)	10.9	1.8	(1.1)	10.1
List.wc ₁	9.51	(3.9)	11.9	2.6	(1.1)	2.9	5.7	(2.0)	11.2	0.25	(0.14)	1.07**
List.wc ₂	9.2	(3.74)	12.1	1.4	(0.56)	3.1	7.0	(3.3)	9.4	0.32	(0.18)	1.2**
List.sup ₁	5.8	(2.4)	5.3**	2	(0.81)	4.1**	3	(1.34)	12.1	0.25	(0.4)	1.1**
List.sup ₂	2	(0.84)	3.0**	2.1	(0.86)	3.6**	5.1	(2.3)	13.5	0.55	(0.32)	1.3**
PPD ₁	7.4	(3.0)	11.8	1.9	(0.79)	3.5**	3.4	(1.5)	11.2	0.2	(0.12)	1.4**
PPD ₂	9.1	(3.7)	14.5	1.30	(0.53)	2.5**	5.9	(2.6)	12.3	0.35	(0.19)	1.3**

(SI): Stimulation Index

(SD): Standard Deviation

(SE): Standard error of the mean

* Number of animals assayed

** significantly different from ground control at .05% level of significance.

ative of the rat response is the data derived in this experiment from a small number of rats. The lymphocytes used in these assays did respond to the specific (when rats were immunized) and non-specific mitogens used. The small number of rats in each group and the wide standard deviations in the data indicates that caution should be exercised in interpreting these results. We were initially concerned with the possibility that the rats immunized with the killed *Listeria* (in Complete Freund's adjuvant) might be poor responders and that we would not be able to demonstrate an in vitro blastogenic response. We did observe, however, blastogenic responses in all of the immunized rats. In this respect the experiment was a success because the immunized rats did manifest a detectable response to the PPD and the *Listeria* antigens. All of the test and control rats also showed a response to the non-specific mitogens PHA and Con A, thereby permitting a valid comparison of flight and ground control rats. Had space flight resulted in a decrease in cell mediated immunity, it would have been detected in this study. Also, the lack of a response of lymphocytes from non-immunized controls to the bacterial antigens is evidence that the rat population from which the experimental rats were selected were free of *Listeria* infection and remained so for the duration of the experiment.

There was a large variation in the response of individual rat lymphocyte cultures to each mitogen (specific and non-specific). Even the unstimulated lymphocytes showed such variation; although not to the same degree as the stimulated lymphocyte cultures. The quadruplicate determinations for each assay also manifested large standard deviations. The response of the flight rats showed extreme variation, however, it is still very evident that the lymphocytes from flight rats were very active and they demonstrated some of the highest incorporations of ^3H -thymidine in response to specific and non-specific mitogens. The poor response of the latter lymphocytes to the *Listeria* supernatant antigen may indicate a difference in antigen

processing or responder and suppressor lymphocyte populations in flight and control rats. The increased blastogenic response of flight rat lymphocytes to *Listeria* whole cells and PPD may indicate that response to whole cells or cell wall antigens may take place in a normal manner and, on the basis of counts/min, ³H-thymidine incorporation, may even be enhanced over ground based controls.

The second most striking result of this study was the impaired response of lymphocytes from synchronous control rats when compared to the response of lymphocytes from ground (vivarium) and flight rats. We have no explanation of this phenomenon except to note that perhaps the stress in a gravity situation was more inhibitory to the development of an immune response than the same type of stress in a weightless environment.

The *Listeria* organism was a good choice as an immunizing agent for the induction of cell mediated immunity. All of the immunized rats responded to this agent. The lymphocyte response to whole cells was better than to the *Listeria* supernatant (sup) and is a reflection of the lack of somatic (cell wall) components in the supernatant preparation.

The choice of an immunizing schedule with *Listeria* and the concentration of blastogenic agents used in assays were empirical. Further studies are required to determine the immunization procedure required for an optimal immune response. We believe that a live replicating vaccine of *Listeria*, containing the native *Listeria* protein, and initiating a short limited infection in the rat spleen, will give a higher immune response than the formalin-killed *Listeria* vaccine, and allow a more sensitive determination of any space flight effect on the induction of thymus dependent cell mediated immunity. Since the question of the safety of live *Listeria* vaccine has not been resolved, further experimentation is required.

The lymphocytes of rats that were flown in a space environment for a period of 19.5 days did not exhibit a decreased blastogenic activity (cellular immune responsiveness)

when compared to ground controls. In addition, those rats subjected to weightlessness demonstrated an increased responsiveness to the blastogenic stimulant, (PHA and Con A) and to the specific Listeria whole cell antigen. There was also a greatly increased responsiveness to PPD, a substance derived from the tubercle bacilli which is used in Complete Freund's adjuvant (in which the Listeria used for immunization was suspended). A comparison of the counts/min of each mitogen giving the best response for each group of flight rats and corresponding control rats (table 5) shows the marked increase in activity of flight rats. The PHA response of the flight rats was, at times, approximately 10X that of the ground controls and 20X that of the synchronous controls. A similar comparison of the activity of the unstimulated flight rat lymphocytes used as a control of the assay consistently show a greater activity than did the lymphocytes from ground controls.

The good PPD response of the flight rats was of particular interest because it was elevated in the flight rats. This finding is of great interest because of the use of BCG to stimulate the immune response for the treatment of certain tumors. If indeed the increased immune response to PPD in space can be confirmed it suggests a practical application of the space environment for immunotherapy of tumors.

This study was suggested by the findings of earlier Soviet space flights in which alterations were reported in the spleen and thymus of flight rats. It is possible that those morphologic abnormalities were not of sufficient magnitude to effect T-cell production and cellular immunity.

These findings fail to support a hypothesis that space flight of 19.5 days may cause a deterioration of cell mediated immunity. The unexpected finding of an increased responsiveness of the lymphocytes of the flight rats should, however, not be viewed as evidence that space flight increased cell mediated immunity, and we can propose no hypothesis to account for this finding. The data, however, all

point in the direction of increased responsiveness in flight rats and strongly suggests that the experiment be repeated when another flight opportunity is available.

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EXPERIMENT K-002

RESULTS OF HISTOLOGICAL EXAMINATION OF INGUINAL LYMPH NODES

SUPPLEMENTARY REPORT by Lisbeth M. Kraft

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Materials and Methods

Inguinal lymph nodes of 18 rats from Experiment K-002 that flew on board Cosmos 782 in 1975 were received in neutral 10% formalin. They were processed for paraffin embedding, and sections 6 μ m thick were stained with hematoxylin and eosin, pyronin-methyl green, and picrofuchsin. Six of the rats were of the vivarium control group, 6 were of the synchronous control and 6 of the flight group.

Results

Lymph nodes of the vivarium control group show only normal variations of structure. Both nodular and diffuse arrangement of the parenchyma can be found, which is further reflected in the fibrous framework as seen in picrofuchsin preparations. Active germinal centers with pyronin positive cells are found in some of the nodes of 3 rats of this group. Mitoses are occasionally observed. Necrotic cells and debris within the centers are normal in amount. Only rarely is a necrotic cell seen elsewhere. The sinuses contain the cells usually seen: lymphocytes, histiocytes, plasma cells, and some erythrocytes.

Essentially the same picture is seen in the synchronous control group, except that occasional foci of necrotic cells can be found in the parenchyma. These are composed at most of only 2 or 3 cells each. Most of the cells seem to be at about the same stage of degeneration. There is no evidence of phagocytosis.

The most outstanding differences between the flight and ground control groups are: 1) marked and widespread depletion of lymphocytes resulting in much larger pale zones than in controls, and 2) the occurrence of numerous foci of pyknotic and necrotic cells together with variable amounts of dust-like debris. The foci are not confined to germinal centers. Rather, they are found throughout the parenchyma as seen in Figs. 1 and 2. Contrary to the situation in the ground control rats (Figs. 3 and 4), active phagocytosis of necrotic cell fragments and debris by fixed macrophages is taking place in the penetrating sinuses of lymph nodes of all flight animals. Further, in some medullary sinuses necrotic cells are also being engulfed by histiocytes, although this is seen far less frequently.

Few necrotic cells can be seen in the subcapsular sinus, indicating that those within the node may be arising there.

Cells with pyronin positive cytoplasm appear to be as numerous in flight animals' nodes as in those of controls.

Comment

The rare necrotic cell seen in the parenchyma of the nodes in the vivarium control animals is regarded as normal. The slight increase of such cells observed in the synchronous controls can be considered a

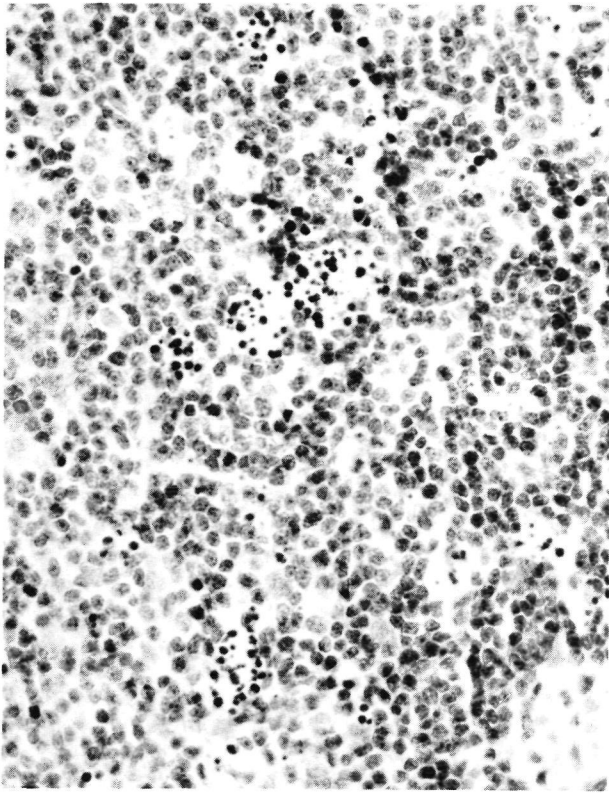


Fig. 1 Flight rat N 4 F. Necrotic cells and debris in a pale zone of the node. Much of the debris appears to be within penetrating sinuses. x440.

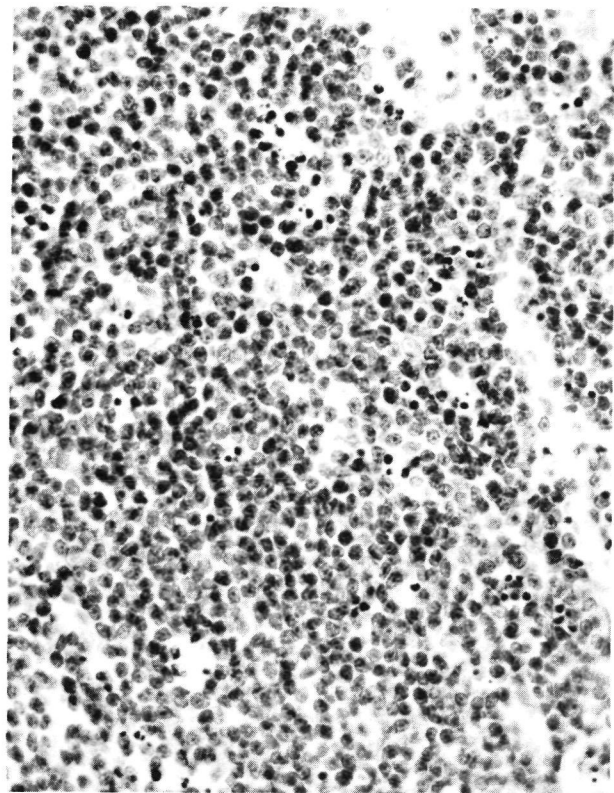


Fig. 2 Flight rat N 4 F. Necrotic cells and debris in a more densely cellular region than in Fig. 1. Scattered individual necrotic cells as well as cell groups are present. x440.

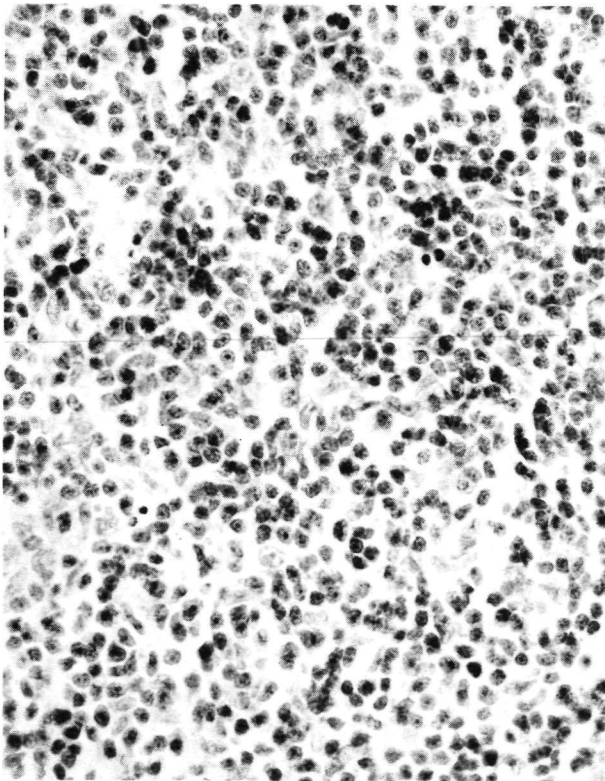


Fig. 3 Synchronous control rat N 3 SC. Pale region of node showing three obvious necrotic cells. X440.

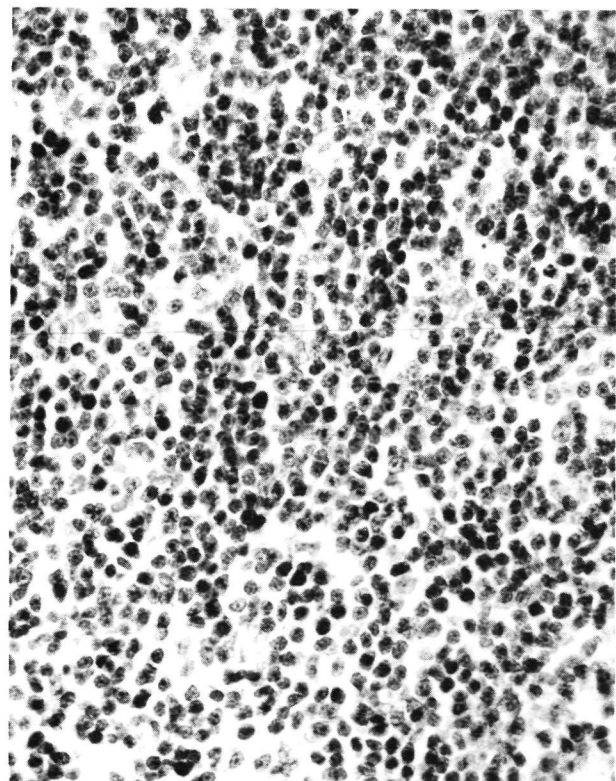


Fig. 4 Vivarium control rat N 5 C. Coronal zone with one necrotic cell. X440.

response to the stress of the simulated landing sequence to which this group had been subjected. The absence of phagocytosis supports this view.

The striking increase in the number of necrotic cells in all nodes of all flight animals, together with evidence of phagocytosis of some cellular debris, is thought to be due to the multitude of stressful conditions of prolonged flight. These would include both weightlessness and cosmic particle radiation, neither of which were experienced by the control groups. Other factors, both known and unknown, might also have played a role.

Precisely how the increased destruction of lymphoid cells fits into the results of the main K-002 experiment or with the increased destruction of erythrocytes shown in Experiment K-003 must remain an open question until further studies can be undertaken.

ALTERATIONS IN ERYTHROCYTE SURVIVAL PARAMETERS

IN RATS AFTER 19.5 DAYS ABOARD COSMOS 782

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ABSTRACT

Rats were subjected to 19.5 days of weightless space flight aboard the Soviet Biosatellite, Cosmos 782. Based on the output of ^{14}CO , survival parameters of a cohort of erythrocytes labeled 15.5 days pre-flight, were evaluated upon return from orbit. These were compared to vivarium control rats injected at the same time. Statistical evaluation indicates that all survival parameters were altered by the space flight. The mean potential life span which was 62.4 days in the control rats was decreased to 59.0 days in the flight rats, and random hemolysis was increased three-fold in the flight rats. The measured size of the cohort was decreased lending further support to the idea that hemolysis was accelerated during some portion of the flight. A number of factors were discussed which might be contributory to these changes. These include, forces associated with launch and re-entry, atmospheric and environmental parameters, dietary factors, radiation and weightlessness. However, their importance relative to the specific conditions of the flight has not been ascertained.

INTRODUCTION

The results of the recent Skylab flights demonstrate that prolonged weightlessness causes a significant decrease in red cell mass of astronauts which is not related to oxygen partial pressure (1). These results are of special interest since a decreased red cell mass was noted in astronauts in some Gemini and Apollo flights (2,3). This was attributed to the pure oxygen environments in use during various phases of the flights, since in Apollo flights where nitrogen was also present, minimal changes were observed (4). On the other hand, Skylab atmospheres contained nitrogen with near normal oxygen partial pressure and yet the red cell mass of the astronauts decreased significantly (1).

Although accelerated hemolysis was considered to be contributory where pure hyperoxic atmospheres were employed, suppression of erythropoiesis has been considered to be the predominant factor overall (1,2,3,4), and accelerated hemolysis was not considered to be a factor when nitrogen was present (1,4). However, there is some experimental evidence from Skylab consistent with the idea that hemolysis was accelerated during the initial stages of the flights (1).

Rats appear to respond to the above parameters in a fashion similar to humans. Thus, rats subjected to twenty-two days of space flight aboard the Soviet Biosatellite Cosmos-605, showed evidence of suppressed erythropoiesis and increased hemolysis (5,6). Likewise, rats show an accelerated hemolysis when exposed to pure hyperoxic environments (7). The main objective of the experiments reported here was to determine if the complex of factors associated with relatively prolonged space flight interact so as to alter survival parameters of preformed red blood cells in rats.

METHODS AND MATERIALS

The technique which we used to study red blood cell survival parameters in this study was developed by S. A. Landaw (8) and is based on quantitation of ^{14}C labeled carbon monoxide exhaled in the breath subsequent to the in vivo labeling of a cohort of red blood cells with 2- ^{14}C glycine. This 2-carbon glycine is rapidly incorporated into 8 positions in the heme molecules of red blood cell hemoglobin and of heme-containing enzymes such as the cytochromes of liver. The 2-carbon of glycine is the unique source of the 4 methene bridge carbon atoms of the heme molecule. During catabolism of the heme portion subsequent to hemolysis, the ring is ruptured at the alpha-methene bridge carbon with the production of carbon monoxide (from this carbon). The degradation of heme in this manner appears to be the sole source of endogenously produced carbon monoxide and it is quantitatively and rapidly excreted in the breath. The turnover of cytochrome heme is very rapid (a few days) compared to that of hemoglobin heme, and therefore the peaks of labeled carbon monoxide arising from the two heme sources are considerably separated by time and therefore can easily be distinguished. Prior to senescence of the red blood cells, which normally survive 62 days in the size and species of rats we used, the rate of labeled carbon monoxide output is proportional to the random hemolysis of red blood cells. As the red blood cells approach senescence, labeled carbon monoxide output increases and then drops off as the cells in this labeled cohort die off. By analysis of the data according to the requisite equations (8), the rate of random hemolysis, the mean potential life span and other survival parameters of a cohort of labeled red blood cells can be determined.

Wistar strain specific pathogen free male rats were injected intraperitoneally with 100 μCi /200 gm of 2- ^{14}C glycine (specific activity 49.73 $\mu\text{Ci}/\mu\text{mole}$) 15-1/2 days before launch. At the time of injection, the vivarium control rats averaged 153 gm and the flight rats averaged 156 gm. (See Figure I). They were 48 days old.

K-003, ERYTHROCYTE SURVIVAL STUDIES

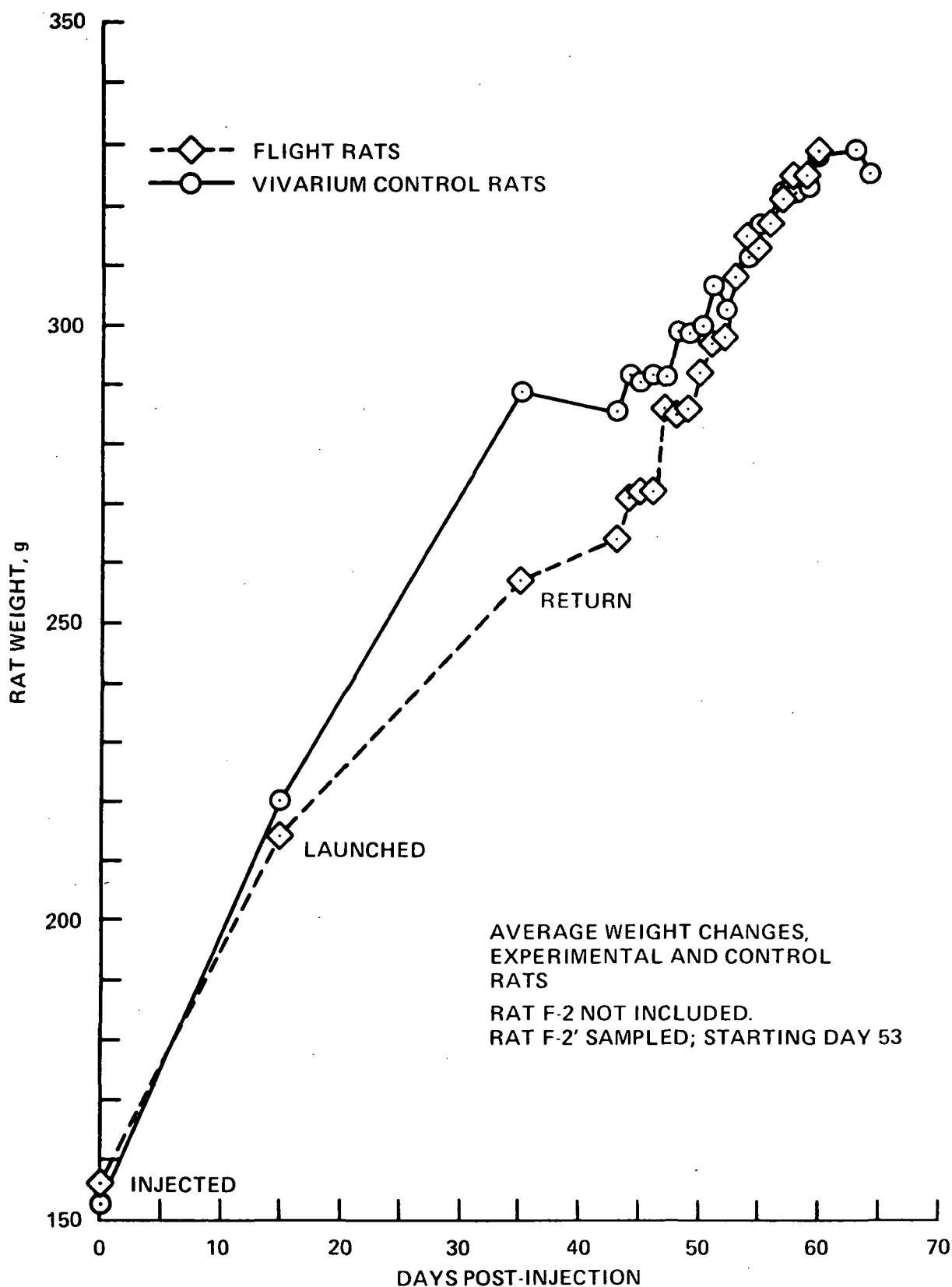


FIGURE 1

A group of synchronous control rats was also injected at this time. These were to be subjected to a simulation of the various stresses, confinements, and feeding schedules experienced by the flight animals. Unfortunately, a malfunction of the simulation apparatus forced a termination of this portion of the experiment.

All rats were fed and watered ad libitum prior to launch, and after recovery from orbit. The vivarium control rats were fed ad libitum during the entire experimental period.

The flight rats were flown aboard the Soviet Biosatellite Cosmos 782 in November/December, 1975, for 19.5 days. Each rat was housed in a special cylindrical capsule 9.5 cm in diameter by 26 cm long with automated feeding and watering devices and a waste management system attached. During flight, the rats were fed a nutritionally balanced paste-like diet. The paste was fed to the rats in 10 gm aliquots, four times a day, and water aliquots were given every 6 hours. The rats were exposed to a 12-hour light, 12-hour dark cycle by means of a light source within the capsule. A more comprehensive description of the flight details has been reported elsewhere (9,10). Upon return from orbit, the flight rats were transported to Moscow, Institute for Biomedical Problems. Six flight rats (F-1 through F-6) and six vivarium control rats (V-13 through V-18) were tested. A total of twenty-three daily points were obtained from the flight rats with the exception of Rat F-2¹. Only 8 points were obtained on this rat, as it was a substitute to flight rat F-2 which died due to unknown causes. The 8 points taken on rat F-2¹ were at the peak of output of radioactive carbon monoxide and therefore sufficient data was available to calculate all parameters. With the vivarium control rats a total of 25 daily data points were obtained. The determinations were started 38 days post-injection of the 2-¹⁴C-glycine (3 days post-flight) and continued through Day 60 post-injection for the flight rats. The vivarium control rats

were tested through Day 64 post-injection.

Quantitation of respiratory ^{14}CO was performed as described previously (7). Briefly, each rat was placed in an individual cylindrical chamber 8.5 x 26.5 cm, and flushed with air at a rate of 600 cc/min. Air exiting from the chamber was dried by passage through anhydrous CaSO_4 . Carbon dioxide was removed by passage of the air stream through soda lime. The ^{14}CO was then oxidized to $^{14}\text{CO}_2$ by a Hopcalite cannister (Mine Safety Appliances Corp., Pittsburgh, Pennsylvania, U.S.A.). The ^{14}CO thus generated was trapped in ethanolamine/2-Ethoxyethanol and triplicate aliquots were counted by liquid scintillation. Breath samples were taken for 3 hours at the same time each day to minimize potential circadian fluctuations.

The activity in ^{14}CO was expressed as dpm/hr. From these data, the following survival parameters relating to a cohort of labeled red blood cells can be obtained:

K, rate of random hemolysis (percent per day).

T, mean potential RBC life span (days).

σ , spread of life spans about T (days).

S, fraction of labeled RBC dying of senescence (%).

$\bar{T}$, mean overall RBC life span (days).

C, fraction of injected glycine incorporated into RBC heme.

In the present experiment, C takes on a different meaning since actual determinations were not made for the initial or final portions of the survival curve. This will be discussed subsequently.

The obtained data points were fitted to an equation relating RBC survival to ^{14}CO production (8), using a Wang 2200B programmable calculator. Initial guesses for each parameter of RBC survival were obtained by visual inspection. In each case, inspection of the final best-fit curves was performed to assure that the

fits were optimal, as documented by a minimization of the sum of the squared differences between the obtained data points and the computer-determined best-fit curve. Data points were rejected if the difference between that point and the best-fit curve exceeded 2 standard deviations. The new set of data points was then re-run until no further outlying data points could be identified.

The optimal running of the above computer program requires a plateau period of ^{14}C production (random RBC destruction), as well as a gaussian-shaped (RBC senescence) portion. The protocol for this experiment did not permit observation of the plateau portion (5-30 days after isotope injection), or of the declining portion of the gaussian peak (64-90 days after isotope injection). Although the final results could theoretically be changed if the entire curves were available for analysis, the null hypothesis was offered that the selected portions of the curves in experimental and control groups should yield identical computer-determined values for RBC survival. That statistically-significant differences were found between the groups strongly suggests that the limited data points do indeed reflect differences in RBC survival.

RESULTS

The ^{14}C output data for the flight and control rats is listed in Tables I and II respectively. The data for both rats F-2 and F-2¹ are included although erythrocyte survival parameters were calculated only for rat F-2. Those data points considered to be spurious since they differ by more than 2 standard deviations from the calculated theoretical values are outlined. Although a number of spurious points may occur on a given day, it can be seen that only two rats had as many as 3 spurious points. These points were sufficiently separated, and the frequency of usable points was such that this was of little consequence in the accurate calculation of erythrocyte survival parameters.

The erythrocyte survival parameters as calculated from the data presented in Tables I and II are presented in Table III. For these calculations, a $T_{1/2}$ of 100 days was used for the glycine recycling component of glycine (8), with a zero-time extrapolate of 15 dph/hour. The values of the flight rats and vivarium controls have been compared without and with the values obtained from rat V-17. Control rat V-17, although having 25 usable daily data points, was an outlier with regard to the calculated erythrocyte survival parameters. In the first case, excluding data from rat V-17, a statistical evaluation using Student's t test indicates that all erythrocyte survival parameters were highly significantly altered by the 19.5 days of space flight. Thus, the mean potential life span, T , which was 63.0 days in the five vivarium control rats was decreased to 59.0 days in the six flight rats ($p < 0.01$). Random hemolysis K , increased more than three-fold ($p < 0.001$) in the flight rats; and the spread of life spans about the mean potential life span (σ) narrowed by three days ($p < 0.001$). Likewise, C , which is a measure of cohort size decreased ($P = 0.01$). The percentage of cells dying from senescence, S , and the average erythrocyte life span T both decreased in the flight rats ($P < 0.001$).

TABLE I

RESPIRATORY ^{14}C O FLIGHT RATS
 ^{14}C O OUTPUT DPM/HR.

<u>DATE</u>	<u>DAYS POST INJECT.</u>	<u>RAT F1</u>	<u>F2</u>	<u>F2¹</u>	<u>F3</u>	<u>F4</u>	<u>F5</u>	<u>F6</u>
12/18	38	31.37	56.68	-	45.41	39.27	61.87	33.74
12/19	39	35.56	55.08	-	39.99	39.45	53.17	30.52
12/20	40	35.62	50.87	-	45.89	44.43	51.94	27.06
12/21	41	21.67	43.44	-	25.54	27.51	35.44	25.16
12/22	42	36.58	51.81	-	31.21	25.31	35.28	29.42
12/23	43	27.40	36.51	-	36.18	36.08	36.93	22.38
12/24	44	31.86	41.66	-	30.94	43.77	47.78	23.31
12/25	45	32.97	44.33	-	39.38	56.07	54.00	29.96
12/26	46	36.94	46.73	-	39.90	57.99	48.14	28.98
12/27	47	34.71	41.94	-	40.41	43.49	43.94	26.50
12/28	48	53.48	60.45	-	54.44	55.64	51.91	39.38
12/29	49	51.45	58.76	-	58.19	63.20	59.68	41.61
12/30	50	63.01	63.19	-	57.74	59.85	70.84	49.51
12/31	51	106.38	147.95	-	129.86	123.78	143.71	105.89
1/1	52	79.19	168.05	-	68.32	80.05	77.94	53.52
1/2	53	102.97	-	96.68	81.12	95.04	85.27	59.69
1/3	54	119.24	-	138.27	102.76	111.27	98.80	84.55
1/4	55	116.96	-	156.20	119.86	128.78	117.31	89.39
1/5	56	162.74	-	191.04	196.20	209.98	148.73	163.78
1/6	57	168.90	-	173.41	186.00	169.62	171.82	122.06
1/7	58	134.35	-	167.97	251.29	182.93	182.06	175.87
1/8	59	168.31	-	207.75	198.38	174.39	175.82	156.76
1/9	60	155.87	-	174.00	170.67	147.04	156.37	190.56

TABLE II

RESPIRATORY ^{14}C O CONTROL RATS ^{14}C O OUTPUT DPM/HR

<u>DATE</u>	<u>DAYS POST INJECT</u>	<u>RAT NO. V13</u>	<u>V14</u>	<u>V15</u>	<u>V16</u>	<u>V17</u>	<u>V18</u>
12/18	38	29.55	26.43	20.17	32.09	29.68	21.68
12/19	39	32.13	29.41	36.74	35.36	33.60	26.14
12/20	40	35.10	36.37	35.33	46.68	36.25	35.71
12/21	41	26.20	21.99	25.08	26.61	23.03	19.29
12/22	42	34.73	27.35	30.27	38.59	38.12	32.06
12/23	43	27.85	27.27	26.94	38.61	29.57	29.22
12/24	44	24.03	25.94	22.69	34.69	23.07	24.80
12/25	45	38.75	36.10	35.20	40.07	27.75	41.63
12/26	46	35.61	29.22	32.32	44.77	22.52	40.31
12/27	47	42.84	44.09	32.98	39.15	23.81	46.66
12/28	48	49.35	51.40	35.85	47.98	28.82	54.42
12/29	49	62.34	55.58	38.22	62.97	35.19	70.54
12/30	50	49.41	42.66	32.52	61.47	38.70	68.97
12/31	51	76.28	65.22	51.14	110.41	95.25	102.75
1/1	52	66.79	76.27	54.81	110.57	86.16	135.05
1/2	53	100.30	92.61	57.17	113.29	76.08	118.82
1/3	54	109.45	104.62	66.31	114.42	82.58	129.04
1/4	55	113.93	105.97	64.91	122.44	98.84	138.56
1/5	56	150.12	130.33	99.53	185.43	146.90	188.89
1/6	57	162.23	130.29	97.43	150.15	86.84	134.13
1/7	58	251.96	153.01	126.15	225.66	141.91	190.20
1/8	59	190.40	154.80	121.61	184.51	110.40	190.87
1/9	60	155.24	108.87	116.27	120.18	68.75	121.24
1/12	63	234.72	182.92	193.89	160.99	86.51	192.54
1/13	64	227.68	195.43	227.09	187.40	99.09	168.42

Table III

RED BLOOD CELL SURVIVAL PARAMETERS

FLIGHT RATS	k	T	σ	C	$\bar{T}$	%S
F1	.91	58.5	4.9	3020	45.4	59.1
F2'	.77	58.5	5.1	3260	47.2	64.0
F3	1.35	58.4	3.7	3495	40.6	45.8
F4	1.31	58.5	4.6	3550	41.1	46.9
F5	1.51	59.0	4.4	3910	39.3	41.4
F6	.77	60.9	4.8	3050	48.7	62.9
MEAN $n = 6$	1.10 ± 0.13	59.0 ± 0.4	4.6 ± 0.2	3381 ± 138	43.7 ± 1.6	53.4 ± 0.4
$\pm S.E.$						
CONTROL RATS						
V13	.41	62.5	6.6	4380	55.2	77.6
V14	.15	63.9	8.9	4100	60.9	90.9
V15	.39	66.6	7.1	4565	58.7	77.3
V16	.41	61.5	7.9	4055	54.4	77.9
V17	.70	59.7	6.2	2330	49.3	69.7
V18	.22	60.5	7.3	3760	56.6	87.7
MEAN $n = 6$	$.38 \pm 0.08$	62.4 ± 1.0	7.3 ± 0.4	3865 ± 327	55.8 ± 1.6	80.2 ± 3.2
$\pm S.E.$						
MEAN $n = 5$	$.32 \pm 0.05$	63.0 ± 1.1	7.6 ± 0.4	4172 ± 139	57.2 ± 1.2	82.3 ± 2.9
$\pm S.E.$						
p VALUE 6 CONTROLS	<.01	<.02	<.001	NS	<.001	<.001
p VALUE 5 CONTROLS	<.001	<.01	<.001	<.01	<.001	<.001

☐ OUTLIER ANIMAL DATA: DO NOT REJECT BY CHAUVENET'S CRITERIA
☒ OUTLIER ANIMAL DATA: REJECT BY CHAUVENET'S CRITERIA

When the data are compared including the data from rat V-17, the differences in the measured cohort size, C, loses its statistical significance. This, of course, is due to the relatively much lower value of C for rat V-17 as compared to the other survival parameters. The pattern exhibited by rat V-17 deserves comment. It was decided initially to introduce the ^{14}C glycine into the rats intraperitoneally rather than intravenously. This greatly facilitates large scale injections and minimizes trauma to the rats. Nevertheless, there is always the risk that some of the glycine may find its way directly into the bladder or the lumen of the intestines and therefore be eliminated without metabolic processing. In such a circumstance, one would expect the value for C to be low whereas all other survival parameters would be unaffected. On the other hand, random hemolysis in this rat is the highest for the control group; this could be an indication that random hemolysis, for reasons unknown, was excessively high in this one control rat prior to the initiation of the measurements. Chauvenet's criterion was applied to the values for the controls (11). For $n = 6$, any point more than 1.73 S.D. from the mean can be rejected. On this basis, C for rat V-17 can be rejected and the p value of C using 5 controls is statistically significant ($p < .01$).

DISCUSSION AND CONCLUSIONS

The results obtained by the method reported here indicate that at least one discrete segment of the erythrocyte population is affected adversely by some aspect associated with space flight. All measured survival parameters are affected, with random hemolysis increased three-fold and the mean potential life span shortened by 5.4%. These evaluations, based on post-flight studies, indicate that the changes impressed upon the red blood cells persisted for at least 25 days post-flight.

Since measurements were not initiated until 3 days post-flight, it is conceivable that some factor associated with re-entry from orbit or some post-flight trauma could cause an alteration in K and T. This contention is not supported by the flatness of the initial pattern of ^{14}CO output nor by the significant decrease noted in C. Normally, when the ^{14}CO output curve is defined in its entirety by actual measurement, C represents the incorporated fraction of the injected glycine and therefore is a measure of actual cohort size. In the present situation, C is calculated on the basis of a trend established after post-injection day 38. Thus, C is a measure of the size of the cohort remaining as of post-injection day 38. On this basis, it can be concluded that about 18% more red blood cells of the labeled cohort were hemolyzed in the flight rats in comparison to the vivarium controls prior to post-injection day 38. ~~These data are consistent with the notion~~ that hemolysis occurred at an accelerated rate in the flight rats at some time prior to post-flight day 3 and probably prior to re-entry. This increased hemolysis, if selective, could account for the decreased mean potential life span, T, and narrowed spread of life spans about the mean, σ , in the flight rats.

There is other evidence suggesting that hemolysis occurs in rats subjected to space flight. Thus, following the 22-day flight of Cosmos 605, the spleens of the flight rats had an increased content of hemosiderin which decreased towards

normal values during the 27-day post-flight study period (6), and there was a decrease in the osmotic stability of erythrocytes on the first day post-flight although no difference in hematocrit or hemoglobin content was noted (5). On the other hand, in the present study of Cosmos 782, no differences in osmotic stability between the flight and control animals were noted (9) and no mention of splenic hemosiderosis has yet been made (12). We must point out, however, that the technique we have employed is a more sensitive detector of hemolysis since it focuses on a discrete portion of the erythrocyte population and also since it is a direct measure of hemolysis the results are minimally influenced by other extraneous variables.

The unfortunate loss of the synchronous control group for this particular study prevents a definitive conclusion concerning the cause of these alterations. Nevertheless, there are a number of factors worthy of discussion and consideration at this time. These include:

1. Forces associated with launch and re-entry, i.e., noise, vibration and acceleration.
2. Atmosphere composition, i.e., O_2 , CO_2 , toxicants.
3. Temperature and humidity.
4. Dietary factors.
5. Radiation.
6. Weightlessness, i.e., mechanisms set into motion by weightlessness.

Although noise and vibration have not been specifically studied, Coltman, et al. (13) have demonstrated that a centrifuge simulation of exit and re-entry acceleration forces in humans ($12 + Gx$ for 30 sec. integrated area under the G-time curve) can cause an immediate increase in the production of endogenous carbon monoxide which is observable for at least two hours post-centrifugation.

Calculations based on the increased CO production, assuming that all of it is hemoglobin derived, would indicate that the daily fractional destruction rate of hemoglobin is increased three-fold. However, the duration of this increased CO production was not ascertained. In all probability, however, it was transient in nature since the data from Skylab do not support the idea of a persistent accelerated hemolysis (1). It is also possible that the source of increased CO was the liver or was an apparent increase caused by an increased post-centrifugation pulmonary ventilation.

Atmosphere composition, particularly oxygen content, is well recognized as a factor associated with altered hemolytic rates. This has been judged to be an important factor in human space flight where pure oxygen environment are employed (2,4). Similarly, a high oxygen partial pressure has been suggested to be the most probable cause of the hemolysis seen in the Cosmos 605 rats (6). In this regard, however, it has been reported the hemolysis caused in rats by pure oxygen, as with humans, is attenuated by the presence of nitrogen (14). Nevertheless, Cosmos 605 had a 3-day period of hyperoxia where the partial pressure of oxygen was between 300 and 390 torr, whereas in Cosmos 782, the O₂ partial pressure never exceeded 253 torr and this was transient.

The CO₂ content in Cosmos 782 never exceeded 8 torr and no report can be found suggesting that CO₂ accelerates hemolysis. With regard to other contaminants it has been demonstrated that exposure of rats to as little as two days in a capsule where urine and feces are allowed to accumulate, but which is otherwise properly aerated, results in a significant increase in erythrocyte osmotic fragility (15).

Temperature and humidity are a consideration in that they affect metabolic rate, body temperature, and food utilization, all of which may affect erythrocyte

life span. Thus, the lesser body weight increase in the flight rats relative to both earth control groups is tentatively attributed, in part, to an increased total motor activity and a resultant increased metabolic rate as indicated by an apparent increase in oxygen consumption during flight in the face of a slightly diminished food consumption (9). This indicated metabolic rate increase could shorten the life span of the erythrocytes, whereas, the diminished average body temperature also reported for the flight rats would tend to have the opposite effect (16).

The increased metabolic rate of the flight rats noted above indicate a greater utilization of constituents supplied directly or indirectly by the diet. This could lead to a deficiency of specific factors which normally contribute to the maintenance of erythrocyte membrane integrity. Two factors to be considered are Vitamin E and cholesterol. Vitamin E deficiency has long been associated with increased erythrocyte hemolysis both in normoxic and hyperoxic environments (17,18). It is of interest to note that a decreased level of plasma Vitamin E possibly associated with an increased utilization has been found in astronauts returning from space flight (3).

Cholesterol in the erythrocyte membrane is reportedly in equilibrium with free plasma cholesterol. If the latter is lowered, membrane cholesterol levels will decrease and this can cause profound but reversible changes in red cell shape which make them osmotically fragile (18,19). It should be noted also that plasma cholesterol has been reported to decrease in some astronauts (3).

Further evidence concerning the importance of cholesterol metabolism has been obtained from lipid studies on humans exposed to pure oxygen environments. Data obtained support the hypothesis that anemia induced by the exposure is caused in part by decreased synthesis of cholesteryl esters secondary to an inhibition of

the plasma lecithin-cholesterol acyltransferase reaction (4).

It has been recognized for some time that ionizing radiation can cause hemolysis by an indirect mechanism. Hemolysis has been reported to occur in cancer patients undergoing X-ray therapy (21). However, hemolysis in dogs, for example, does not become evident until near lethal doses of radiation (300-385) are used (22). It must be pointed out, however, that the criteria used to assess hemolysis in these situations was less sensitive than the technique we have employed in the present work. The results of the recent Skylab flights demonstrate that prolonged weightlessness causes a significant decrease in red cell mass. This decrease measured upon return to earth was most severe in the shorter 28-day Skylab mission and least in the 84-day flight (1). One interpretation of this is that RBC mass was equally and severely affected in all three flights by some initial condition, i.e., fluid shifts; but in the longer flights, there was sufficient time for adaptation and training to cause a partial restoration of the RBC mass (23). Present experimental data from Skylab indicate that the initial conditions of space flight cause a reduction in the red cell mass by an initial shutdown of erythropoiesis and a possible accelerated hemolysis of the older red blood cells (1). This may be a simple expression of a decreased requirement. The shutdown appears to be caused by a hemo-concentration caused either by auto-transfusion and/or plasma volume contraction (3). In regard to the latter possibility, there is no evidence indicating an initial diuresis (24). How accelerated hemolysis could occur is not clear, but one may speculate that the spleen, in the face of hemo-concentration, is programmed in some fashion to select out and hemolyze the older red blood cells. Initial fluid shifts, net fluid loss and/or autotransfusion could cause a hemo-concentration with a resultant increase in viscosity and a decrease in oxygen transport capacity. This, in turn, could trigger mechanisms which cause a seques-

tration and subsequent hemolysis of red blood cells. In this regard, it has been suggested that hemolysis of the older erythrocytes is a possibility (1).

In conclusion, a number of variable factors have been considered and discussed which may by themselves or in combination cause the hemolysis and shortened life span of the labeled cohort of erythrocytes seen in the Cosmos 782 flight rats. Most of these factors are not unique to the space environment. However, their importance relative to the specific conditions of the flight have not been ascertained. Nevertheless, there is evidence to support the view that weightlessness can initiate changes which lead to the alterations reported here.

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EFFECTS OF SPACE FLIGHT ON PLASMA AND GLANDULAR
CONCENTRATIONS OF PITUITARY HORMONES

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ABSTRACT

Pituitary function was investigated in rats subjected to 19.5 days orbited space flight. Male SPF Wistar rats were divided into vivarium control (VC), synchronous control (SC), and flight (F) groups. SC rats were subjected to the same caging, pO_2 , pCO_2 , and temperature as F rats. Rats from each treatment group were sacrificed either immediately after recovering from flight (R+0) or 25 days after recovery. Space flight caused a marginal inhibition of growth. Flight rats weighed less than SC at R+0 and less than VC at R+25. They also had shorter tails than VC at R+0 and R+25. At R+0 F rats had lower plasma GH titers than SC and VC and lower ADH levels than SC; plasma concentrations of ACTH, prolactin, TSH, FSH, LH, and MSH did not differ from controls. Pituitary concentrations of the aforementioned hormones were similar for all groups as were the hematocrits. At R+25 F rats had decreased plasma prolactin concentrations, decreased pituitary GH and increased pituitary vasopressin; pituitary and plasma concentrations of the other hormones remained unchanged from control values. Hematocrits of flight rats were higher than VC and SC values at R+25 and higher than for F rats at R+0. Anterior pituitary and testicular weights were unaffected by space flight, whereas adrenal weights (2 rats from each group) were 30% heavier than controls at R+0 and 15% heavier at R+25. Flight rats also had enlarged posterior lobes.

INTRODUCTION

During space flight men and animals experience several metabolic changes. Some of these changes, such as protein and calcium losses, appear to be detrimental to the organism and do not abate with continued space flight, thereby posing a potential limit to the duration of manned space flight. Astronauts and cosmonauts have sustained losses of body water, sodium, potassium, phosphate, calcium, and protein (1,2,3,4). Dogs and rats have shown losses of bone and muscle mass suggesting metabolic responses to space flight analogous to those of man (5,6). Bed rest simulation of space flight has also evoked metabolic responses qualitatively similar to those of actual space flight (7,8).

The important role played by hormones in regulation of the metabolic parameters affected by space flight suggests that altered endocrine function may have contributed to the observed metabolic changes. In previous studies of space flight-related endocrine function, pituitary hormones, parathormone, insulin, renin activity (angiotensin I generation), adrenal steroids, thyroid hormones, and catecholamines, have been measured in plasma and/or urine in flight, immediately post-flight, or during simulated flight (bed rest) (1-4, 7,8). The only consistent findings have been decreased plasma insulin after 19.5 days flight and elevated plasma levels of insulin, growth hormone, aldosterone, thyroxine, and thyrotropin post-flight. During flight urinary aldosterone and cortisol increased whereas vasopressin excretion decreased the second and third month of flight. Rats have shown increased plasma corticosterone at recovery (5). In human subjects, cortisol and renin have not shown statistically significant changes in plasma concentrations from preflight values. It is evident that an unequivocal assessment of space flight effects on endocrine function has not emerged thus far. The causes for this equivocation are, in part at least, the limitations of sampling conditions and numbers of samples, the

transitory nature of plasma hormone concentrations under less than standard conditions, and, in some instances, the complicating effects of re-entry.

The aims of the present study were to clarify the effects of space flight on pituitary function by measuring plasma and glandular concentrations of pituitary hormones and, where feasible, evaluating the status of the appropriate target tissue.

METHODS AND PROCEDURES

The rats, diet, and maintenance conditions have been described elsewhere, as have relevant conditions of flight and recovery (9). For unrelated experiments the rats were given ^{14}C -glycine (100 μCi /200g B.W.) i.p. at -15 to -5 days, *Listeria Monocytogenes* vaccine in the foot pads at -5 days, and Declomycin (1 mg/kg B.W.) i.p. at -3 days. A preliminary study indicated that at the dosages used, these treatments had negligible effects on endocrine function.

At the conclusion of the flight all rats were weighed and inspected. Six rats from each treatment group were decapitated and their tail lengths measured. Trunk blood was collected into heparinized tubes, centrifuged at 1500 x g for 20 min. at 5°C and the plasma frozen for hormone assays. Anterior and posterior-intermediate lobes were collected, weighed on a Cahn Micro Balance and individually frozen for assays. Adrenal glands were collected from 2 rats of each group, trimmed of extraneous tissue, weighed and placed in 10% neutral formalin solution. One testicle from each rat was weighed. The balance of flight and control animals were sacrificed 25 days after flight and samples collected as detailed.

Frozen or fixed samples were delivered to Ames Research Center for further processing and assays of growth hormone, prolactin, and vasopressin. Assays of gonadotropins and thyrotropins were performed at the University of California, Los Angeles; adrenocorticotropin assays were done at the Veterans Administration Hospital, Portland, Oregon, and melanocyte stimulating hormone assays at the University of California, Davis. Anterior pituitary glands were homogenized in all glass or glass and teflon hand homogenizers in chilled 0.01M Na_2CO_3 , pH 10.5, at a concentration of 1 mg tissue (fresh weight) per ml. One ml of each homogenate was immediately added to 1 ml chilled 0.2 M HCl for radioimmunoassay (RIA) of adrenocorticotrophic hormone; the final pH of the acidified homo-

genates was 1.12 - 1.45. One ml aliquots of alkaline homogenates from all samples of a given treatment group were pooled for tibial assay of growth hormone (10). The remaining homogenate was used for RIA of the other anterior lobe hormones. Posterior-intermediate lobes were similarly homogenized in chilled 0.1 M HCl and assayed for vasopressin and melanocyte stimulating hormone (MSH).

Plasma and pituitary growth hormone (GH) and prolactin were immunoassayed by the methods of Schalch and Reichlin (11) and Niswender, et al. (12) respectively, using GH and prolactin purified inhouse as standards. Thyrotropin was assayed by a double antibody method (Parlow, A. F., unpublished) and luteinizing and follicle stimulating hormones by the procedure of Daane and Parlow (13) employing NIAMDD rat standards. ACTH was immunoassayed by the method of Rees, et al. (14) using the 3rd International Standard (porcine). Vasopressin was measured by the RIA procedure of Keil and Severs (15) and MSH by the bioassay method of Geschwind and Huseby (16).

RESULTS

Body and Organ Weights:

The smaller body weights of flight rats compared to those of synchronous controls at R+0 and vivarium controls at R+25 and their shorter tails suggested that space flight had inhibited growth. (Tables 1,2). However, the inhibition of growth is, at most, marginal as flight rats were not smaller than both control groups at both times of sacrifice and the absolute differences in weight were only 15g (R+0) and 10g (R+25) or about 6% of body weight in each case. Moreover, Asling (22) found that tibial lengths of flight rats were not shorter than those of control animals.

Posterior pituitaries of flight rats were larger than those of synchronous and vivarium controls at R+0 when expressed as a function of body weight (Table 3). Adrenal glands of flight animals also appeared to be enlarged although the availability of glands from only two rats in each group precluded statistical evaluation (Table 4). Adrenals of flight rats weighed 33% and 25% more than those of vivarium and synchronous controls, respectively, at R+0; at R+25, flight adrenals were about 15% bigger than those of control groups. Similar differences in adrenal weight were found when expressed as a fraction of body weight. Neither testicular nor anterior pituitary weights showed significant differences between treatment groups. Hematocrits of all animals were similar at R+0 whereas at R+25 the hematocrit of flight rats was significantly higher than that of VC and SC rats and of flight rats at R+0 (Table 5).

TABLE 1
FINAL BODY WEIGHTS, g

MEAN $\pm$ STANDARD ERROR

	VIVARIUM	SYNCHRONOUS	FLIGHT
RECOVERY + 0			
SAMPLE DONORS	270 $\pm$ 5 (6) ¹	273 $\pm$ 6(6)	260 $\pm$ 3(6)
ALL RATS	267 $\pm$ 4(16)	273 $\pm$ 4(12)	258 $\pm$ 2(12) ²
RECOVERY + 25			
SAMPLE DONORS	328 $\pm$ 6(5)	324 $\pm$ 5(5)	331 $\pm$ 6(5)
ALL RATS	340 $\pm$ 6(11)	340 $\pm$ 9(11)	322 $\pm$ 5(11) ³

¹NUMBER OF RATS

²DIFFERS FROM SC ($p < 0.05$)

³DIFFERS FROM VC ($p < 0.05$)

TABLE 2
TAIL LENGTHS, mm
MEAN $\pm$ STANDARD ERROR

	VIVARIUM	SYNCHRONOUS	FLIGHT
RECOVERY + 0(6) ¹	171.5 $\pm$ 3.7	158.5 $\pm$ 0.5 ²	157.8 $\pm$ 2.8 ²
RECOVERY + 25(5) ¹	175.8 $\pm$ 1.4	171.0 $\pm$ 1.9	169.3 $\pm$ 1.5 ²

¹NUMBER OF ANIMALS/GROUP

²DIFFERS FROM VC ($p < 0.02$)

TABLE 3
POSTERIOR PITUITARY WEIGHTS, mg/100 g

MEAN $\pm$ STANDARD ERROR

	VIVARIUM	SYNCHRONOUS	FLIGHT
RECOVERY + 0(6) ¹	0.37 $\pm$ 0.01	0.35 $\pm$ 0.01	0.44 $\pm$ 0.02 ¹
RECOVERY + 25(5) ¹	0.37 $\pm$ 0.02	0.35 $\pm$ 0.01	0.39 $\pm$ 0.03

¹NUMBER OF ANIMALS/GROUP

TABLE 4
MEAN ADRENAL WEIGHTS

	VIVARIUM	SYNCHRONOUS	FLIGHT
RECOVERY + 0(2)¹			
mg	16.3	17.3	21.5
mg/100mg	5.95	6.09	8.13
RECOVERY + 25(2)¹			
mg	20.3	19.6	23.9
mg/100mg	6.43	6.10	7.17

¹NUMBER OF ANIMALS/GROUP

TABLE 5 HEMATOCRITS

MEAN $\pm$ STANDARD ERROR

	VIVARIUM	SYNCHRONOUS	FLIGHT
RECOVERY $\pm$ 0(6) ¹	57.5 $\pm$ 2.6	58.1 $\pm$ 2.3	54.0 $\pm$ 1.2
RECOVERY + 25(5) ¹	53.2 $\pm$ 1.6	53.0 $\pm$ 1.5	60.8 $\pm$ 1.3 ²

¹NUMBER OF ANIMALS

²DIFFERS FROM SC AND VC ($p < 0.01$)

Hormone Assays:

Flight rats had lower plasma GH titers than either control group at R+0 Table 6. At R+25 there were no differences between groups and the flight and synchronous values showed an unusual dispersion with concentrations as high as 58 $\mu\text{g}/\text{ml}$. Determinations of pituitary GH by RIA from the mean of individual samples or aliquots of pooled sample gave comparable values in all cases, but the unusual finding was the invariably higher values by RIA than by tibial assay. For the two largest discrepancies, R+0 synchronous and R+25 vivarium rats, duplicate bioassays gave virtually identical values. Previous studies in our laboratory have always yielded equal or higher concentrations of pituitary GH by bioassay than RIA (17). The concentrations of GH in all rats sacrificed at recovery were higher than those sacrificed 25 days later; total pituitary GH also decreased in the interval for all groups. At R+25 pituitaries of flight rats had slightly lower concentrations of GH than those of synchronous or vivarium rats and significantly less total GH than synchronous pituitaries. Czechoslovakian rats kept at Ames Research Center, although larger and older than those used for flight studies, showed the usual ratios of bioassayable to immunoassayable pituitary GH and no change by tibial assay or an increase by RIA in total GH (Table 7).

At recovery, flight and synchronous rats had mean plasma prolactin concentrations of 1.8 $\mu\text{g}/\text{ml}$, which did not differ from those of vivarium rats (Table 8). Flight rats also had low prolactin titers at R+25 which were significantly less than for both control groups.

Plasma ACTH levels did not vary significantly between groups at either R+0 or R+25 and all were above the levels usually found in unstressed animals (18). The plasma ACTH concentrations of flight rats were marginally ($0.1 p > .05$)

TABLE 6
GROWTH HORMONE

	INDIVIDUAL SAMPLES (RIA)			POOLED ANTERIOR PITUITARY TISSUE (MEAN $\pm$ STANDARD ERROR)			
	PLASMA	ANT. PITUITARY		RIA		TIBIAL ASSAY	
	m μ g/ml	μ g/mg	μ g/GLAND	μ g/mg	μ g/GLAND	μ g/mg	μ g/GLAND
RECOVERY + 0(6)¹							
VIVARIUM	7.3 $\pm$ 1.8	52.5 $\pm$ 2.7	265 $\pm$ 13	54.4	275	52 (46-60) ²	265 (234-305) ²
SYNCHRONOUS	7.2 $\pm$ 2.3	51.9 $\pm$ 2.5	242 $\pm$ 25	55.1	254	45 (40-50)	205 (183-231)
FLIGHT	2.0 $\pm$ 0.1 ¹	50.0 $\pm$ 2.1	254 $\pm$ 16	50.4	256	49 (44-54)	248 (225-276)
RECOVERY + 25(5)¹							
VIVARIUM	4.5 $\pm$ 1.8	37.2 $\pm$ 2.0	228 $\pm$ 22	38.0	230	31 (27-34)	185 (162-209)
SYNCHRONOUS	24.6 $\pm$ 12.1	38.3 $\pm$ 2.1	252 $\pm$ 20	38.3	250	35 (31-39)	225 (201-251)
FLIGHT	11.9 $\pm$ 7.1	32.9 $\pm$ 1.0	197 $\pm$ 7 ⁴	34.4	206	33 (29-37)	196 (175-220)

¹NUMBER OF ANIMALS/GROUP

²95% CONFIDENCE LIMITS

³DIFFERS FROM APPROPRIATE SC AND VC VALUE ($p < 0.05$)

⁴DIFFERS FROM APPROPRIATE SC VALUE ($p < 0.05$)

TABLE 7
PITUITARY GROWTH HORMONE OF CZECHOSLOVAKIAN
RATS KEPT AT AMES RESEARCH CENTER

	DATE SACRIFICED	
	11/3/75	2/25/76
n	4	4
MEAN BODY WEIGHT, g	353.0	500.5
MEAN ANTERIOR PITUITARY WEIGHT, mg	7.0	7.2
GROWTH HORMONE		
RADIOIMMUNOASSAY		
$\mu\text{g}/\text{mg}$	26.0	29.2
$\mu\text{g}/\text{GLAND}$	181.2	209.7
TIBIAL ASSAY		
$\mu\text{g}/\text{mg}$	29.1	29.1
$\mu\text{g}/\text{GLAND}$	202.8	208.9

TABLE 8
HORMONE CONCENTRATIONS IN PLASMA

MEAN $\pm$ STANDARD ERROR

	PROLACTIN (m μ g/ml)	ADRENOCORTICOTROPIN (pg/ml)	MELANOCYTE STIMULATING HORMONE UNITS/ml	THYROTROPIN (m μ g/ml)	VASOPRESSIN (pg/ml)
RECOVERY + 0(6)¹					
VIVARIUM	6 $\pm$ 1.9	278 $\pm$ 46	~3	278 $\pm$ 42	3.1 $\pm$ 0.7 ²
SYNCHRONOUS	1.8 $\pm$ 0.4	267 $\pm$ 53	~3	403 $\pm$ 53	5.4 $\pm$ 0.6
FLIGHT	1.8 $\pm$ 0.7	170 $\pm$ 22	~3	224 $\pm$ 36 ²	1.7 $\pm$ 0.6 ²
RECOVERY + 25(5)¹					
VIVARIUM	16.4 $\pm$ 5.9	331 $\pm$ 58		459 $\pm$ 54	2.5 $\pm$ 0.4
SYNCHRONOUS	8.8 $\pm$ 1.0	298 $\pm$ 45		360 $\pm$ 30	2.8 $\pm$ 0.5
FLIGHT	2.0 $\pm$ 0.9 ²	303 $\pm$ 41		211 ³	3.3 $\pm$ 0.3

¹NUMBER OF ANIMALS/GROUP

²DIFFERS FROM SC (p < 0.05)

³ONE SAMPLE ONLY

less than those of vivarium rats and total pituitary ACTH of synchronous rats was significantly less than for the vivarium group at R+0 (Tables 8, 9).

Plasma and pituitary concentrations of melanocyte stimulating hormone did not differ between any of the groups.

Synchronous rats had plasma thyrotropin levels significantly higher than those of flight rats at R+0 and more pituitary thyrotropic hormone at R+25 than either flight or vivarium animals. Pituitary concentrations and content of the other glycoprotein hormones, luteinizing and follicle stimulating hormones, showed no differences between any of the groups (Table 9). At recovery, synchronous control rats had higher plasma vasopressin titers than either vivarium or flight animals, but their level decreased by R+25 so that differences between groups disappeared. At R+25 days, however, the pituitary content of vasopressin was greater in flight than synchronous rats (Table 8, 9).

TABLE 9
ANTERIOR PITUITARY HORMONE CONTENT

MEAN ± STANDARD ERROR							
	PROLACTIN (μg/GLAND)	ADRENOCORTICO- TROPIN (mμg/GLAND)	MELANOCYTE STIMULATING HORMONE (UNITS/mg TISSUE)	THYROTROPIN (μg/GLAND)	LUTEINIZING HORMONE (μg/GLAND)	FOLLICLE STIMULATING HORMONE (μg/GLAND)	VASOPRESSIN (μg/GLAND)
RECOVERY + 0(6) ¹							
VIVARIUM	25 ± 4	874 ± 150	5300 ± 640	661 ± 84	111 ± 5	56 ± 3	2.3 ± 0.2
SYNCHRONOUS	19 ± 2	470 ± 60 ²	4030 ± 500	640 ± 34	94 ± 9	47 ± 4	1.9 ± 0.2
FLIGHT	27 ± 4	667 ± 69	5130 ± 810	633 ± 29	114 ± 26	42 ± 6	2.4 ± 0.3
RECOVERY + 25(5) ¹							
VIVARIUM	39 ± 3	996 ± 86		731 ± 80 ³	122 ± 21	73 ± 8	2.5 ± 0.2
SYNCHRONOUS	34 ± 1	1166 ± 180		995 ± 78	152 ± 18	83 ± 10	2.2 ± 0.1
FLIGHT	40 ± 3	2103 ± 573		756 ± 62 ³	128 ± 21	76 ± 11	2.9 ± 0.2 ³

¹NUMBER OF ANIMALS/GROUP

²DIFFERS FROM VC (p < 0.05)

³DIFFERS FROM SC (p < 0.05)

DISCUSSION

The effects of space flight on pituitary function appear to be few in number, of limited severity, and compatible with the essential well being of the organisms in-flight and post-flight. Interpretation of the present data must be tempered by 1) the small number of animals in some cases (e.g., adrenal weights of 2 rats), 2) the possible complicating effects of re-entry on plasma hormone levels, and 3) the lack of quantitative data on the metabolic status of the animals. It is entirely possible that small changes in endocrine function, undetected in the present study, could lead to serious metabolic problems in flights of longer duration. Although of modest extent, some perturbations of endocrine function were found in this investigation.

The low plasma GH titers of flight animals at R+0 either reflects the unusual sampling conditions at recovery or an effect of space flight. The inhibition of plasma immunoreactive GH levels in the rat by a variety of stresses has been well established and raises the possibility that the stresses of re-entry and recovery inhibited GH secretion (18). Grindeland, R., unpublished observations). However, synchronous rats were subjected to manipulations and, presumably, stresses comparable to those experienced by flight rats during re-entry. The lower plasma GH concentrations of flight rats would, therefore, seem due to other causes such as weightlessness. The flight rats grew well after recovery but their lower pituitary content of GH provides further suggestion that space flight altered GH secretion. The larger adrenals of flight animals suggests that they were secreting increased quantities of corticosterone over a significant part of the flight which may have contributed to the smaller body weights of flight animals by its protein catabolic effect.

Losses and shifts of body water have been observed in actual or simulated space flight of men (1-4, 7,8) and dehydration in space flight animals has been

reported (5,6). The body water content and distribution in Cosmos 782 rats is unknown but the low vasopressin and prolactin titers of flight animals do not indicate a significant loss of body water. Similar hematocrits of all groups at R+0 is consistent with the view that body water was not lost. At R+25 flight rats continued to have circulating prolactin and vasopressin levels in the low to normal range whereas their hematocrits were increased. If the higher hematocrits were due to water loss, the responses of the hormones is inappropriate and implies that disturbance of water balance regulation has occurred. Elevated thyrotropic hormone and thyroxine have been found in post-flight cosmonauts (4) and astronauts (2,3) whereas in this study only synchronous controls had elevated plasma thyrotropin levels at recovery. The reasons for the disparate post-flight thyrotropin responses of men and rats is not apparent.

Elevated plasma ACTH levels revealed all groups of rats were stressed at the times of sacrifice, most probably due to the conditions of sacrifice (14). Elevated ACTH concentrations resulting from the stress of sacrifice may have obscured any differences between treatment groups. The adrenal hypertrophy of flight rats at the time of recovery certainly suggests that these animals were secreting more ACTH and corticosterone than controls during the time of flight. While the modest hypertrophy seen at R+25 is most likely a residual effect of space flight it is also possible that it reflects some stress associated with adapting to 1g; rats centrifuged at 2.7 or 4.1 x g show adrenal hypertrophy for the first several weeks of centrifugation (Keil, L.C., unpublished observations).

Plasma and pituitary melanocyte stimulating hormone concentrations show increases and decreases, respectively, in response to a variety of, but not all, physical and psychological stresses (19,21). In the present study, there were no

changes in either pituitary or plasma MSH suggesting that either the type or degree of stress was not adequate to stimulate MSH secretion (20). These studies have demonstrated that some alterations in pituitary function occurs in response to space flight. The relative contributions of weightlessness, flight-related stress, and long-term effects of these changes remain to be investigated.

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HISTOLOGICAL STUDIES ON TIBIAL BONE
OF RATS IN THE 1975 COSMOS-782 FLIGHT

Part 1. Endochondral Osteogenesis; Medullary Bone Turnover

by

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ABSTRACT

Tibia lengths, histological appearance of the proximal tibial epiphysis and metaphysis, and measurements of the bony spongiosa in the latter, have been reported for 6 male rats subjected to weightlessness in earth-orbit for 19 days, and compared with similar studies conducted on vivarium controls and on synchronous controls (rats experiencing identical conditions except for orbital flight).

Growth retardation was not marked, falling between a negligible level and 25%. Bone formation was slightly impaired in synchronous controls, and to an appreciably greater extent in flight animals. Bone resorption was moderately accelerated in synchronous controls, markedly more so in flight animals, to an extent under which virtually all pre-flight medullary bone was removed and in addition a substantial fraction ($\frac{1}{4}$ - $\frac{1}{2}$) of that formed during the flight was also resorbed.

Although the extent of change in the synchronous controls indicates that disuse atrophy and other restrictions during the experiment account for a part of the imbalance, the condition of weightlessness added a considerable further imbalance which cannot be attributed to such disuse atrophy alone.

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INDEXING

Bone resorption

Medullary bone turnover

Hypogravity

This report concerns the histological evaluation of endochondral osteogenesis in rats of the COSMOS experiment of November - December, 1975. Balances between cartilage formation and resorption, and medullary bone formation and resorption are analyzed in the proximal segment of the tibia.

METHODS

Bones were available from each of three groups of rats (6/group) which had been sacrificed at the time of recovery of the satellite vehicle, including Flight group (FO), Synchronous controls (SO), and Vivarium controls (VO). The tibia of each rat had been removed, stripped of the major part of surrounding skin and muscle, and frozen (unfixed) on dry ice, usually within 20 minutes (15 - 43 minutes) after death. Bones were packaged in sealed plastic envelopes and kept frozen during subsequent transport.

When bones were received, six days later, the proximal and distal articular surfaces were trimmed down to bone and the length measured with vernier calipers to 0.1 mm. accuracy. Bones were then sawed transversely with a jeweler's saw at one-third the distance from the proximal end. The proximal segment was fixed by thawing in chilled 10% formalin in normal saline solution, neutralized with magnesium carbonate. The distal segment was kept frozen for other studies. Fixative was changed after 24 hours at room temperature. After 48 hours the proximal segment was further divided by jeweler's saw by (a) removing 3-4 mm. of shaft transversely (for the microradiographic studies described in Part 2. of this study) and (b) dividing the remainder into two pieces in the sagittal (antero-posterior) plane. The cut surface thus displayed the proximal tibial epiphysis, its epiphyseal cartilage plate, the underlying metaphysis

and medullary cavity, and a short segment of diaphyseal cortex. In the division one piece was cut slightly larger than the other, and was decalcified in 5% aqueous solution of nitric acid, neutralized in 5% sodium sulphate solution, washed, upgraded through alcohols, embedded in celloidin, sectioned at 8 microns thickness, stained with (a) hematoxylin and eosin, and (b) Mallory's triple stain (aniline blue, orange G, and acid fuchsin), and studied histologically. The smaller counterpart piece was reserved, undecalcified, for study of thin ground sections by ultraviolet light.

Condition of specimens

The freezing and delayed fixation (which, as mentioned earlier, was a necessary compromise to provide maximal opportunity for study by other groups of workers) expectedly resulted in inadequate preservation of marrow cells, osteoblasts, osteoclasts, and chondrocytes. (Ice crystals thus formed are disruptive of cell membranes, and partial cytolysis can take place which not only obscures structure but alters stainability of adjacent tissues.) Also, in a small number of instances (especially in the FO series) bone fragility and the rigorous circumstances of collection had led to subepiphyseal fracture or cortical splintering. However, the material was quite adequate for histological identification and measurement of cartilage, bone, and marrow regions.

RESULTS

Measurements were made wherever possible to provide quantitative support for the qualitative histological evaluations. Means and standard errors, corrected for small size of sample, are given in Table 1. The photomicrograph of a Vivarium control given as Figure 1 has an associated legend which identifies some of the structures studied.

Table 1
Gross and Histological Measurements of
Ossification at the Proximal End of the Tibia
(Means and Standard Errors)

Parameter Measured	VO Vivarium Controls	SO Synchronous Controls	FO Flight Group
Tibia length, mm.	38.8 ± 0.31	37.7 ± 0.16	38.4 ± 0.48
Proximal epiphyseal cartilage plate thickness, microns	218 ± 12.3	202 ± 8.7	199 ± 5.6
Primary spongiosa length, microns	817 ± 21	547 ± 48	315 ± 24
Primary spongiosa abundance, No./mm. bone breadth	14.9 ± 0.98	16.2 ± 0.87	12.2 ± 1.21
Primary spongiosa proportion in bone breadth, %	46.0 ± 3.21	38.5 ± 3.57	39.5 ± 3.34
Primary spongiosa, average breadth in microns	31.3 ± 2.11	24.8 ± 3.28	33.0 ± 2.63
Entire spongiosa, depth in medullary cavity, mm.	2.93 ± 0.20	1.34 ± 0.18	0.97 ± 0.22



- - - Bony rim of epiphyseal ossification center
- EPIPHYSEAL CARTILAGE PLATE
- - - Line of erosion of plate
- - ↘
- PRIMARY SPONGIOSA (bone)
Delicate, abundant, often showing inclusions of pale-staining cartilage matrix
- - ↘ Region of transition to
- SECONDARY SPONGIOSA (bone)
Sturdy, less abundant, seldom showing cartilage matrix inclusions

Figure 1. Vivarium control (VO 6)



Figure 2. Synchronous control (SO 2)

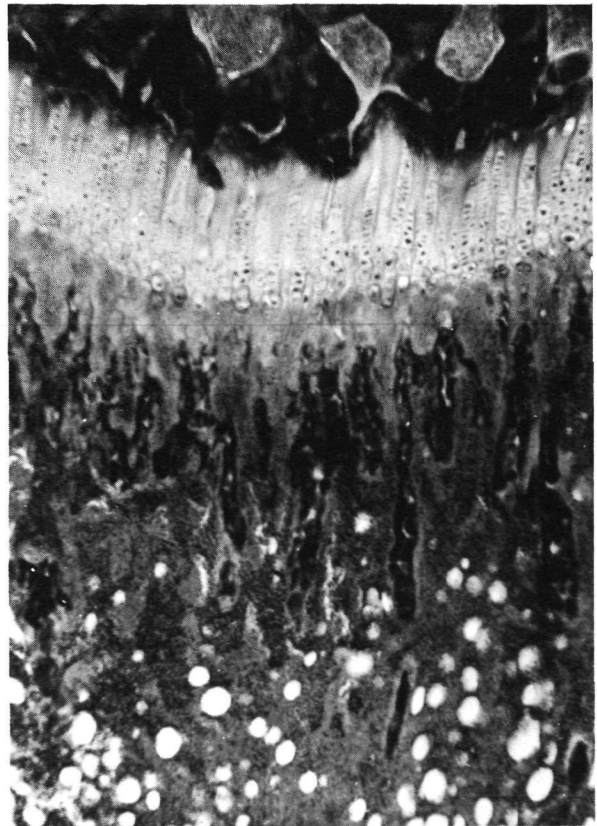


Figure 3. Flight group (FO 1)

Tibia length. With a single exception, no significant difference was found among mean tibia lengths in any group. Synchronous controls were slightly shorter than Vivarium controls; however, since substitution had been necessary in the Synchronous control group, the difference cannot be attributed to the primary experimental circumstances.

Epiphyseal cartilage plate thickness. This parameter is an indicator of the balance between the rate of formation of cartilage (chondrogenesis) and the rate of its removal and replacement by endochondral bone during growth of the bone in length. It was measured at a microscope magnification of 75, with an ocular micrometer calibrated to read 1 scale division = 13.6 microns. Measuring points were between the bony rim of the epiphyseal ossification center and the line of cartilage erosion represented by the advance of marrow tufts and capillaries at the diaphyseal aspect of the cartilage plate (Fig. 1). Five such readings were made on each specimen, in the middle 50% of the breadth of the cartilage, and the average was used as the value of cartilage thickness for that animal.

As shown in Table 1, no significant difference appeared among any of the groups; mean thickness was close to 200 microns in both Synchronous control and Flight animals, and slightly (but insignificantly) greater in Vivarium controls. The similarity can be judged by comparison of the photomicrographs in Figures 1, 2, and 3, in which the section most closely approximating the mean was selected for illustration of each group.

Examination of the regions within the epiphyseal cartilage plate likewise showed no special histological characteristics distinguishing any one group from another.

Bone: Primary spongiosa.

Vivarium controls: the appearance was in accord with that expected in post-puberal animals undergoing a moderate rate of bone growth (Figure 1). Sturdy bone trabeculae were formed on cores of cartilage matrix which was continuous with the matrix of the epiphyseal cartilage plate. Osteogenesis started within a few microns of the zone of cartilage erosion, and the bony trabeculae continued well into the diaphyseal marrow cavity with abundant anastomoses between adjacent trabeculae. From thence many of them were continuous with the trabeculae of secondary spongiosa; a few were truncated with the sharply-cut tips which characterize osteoclastic activity necessary in the removal of these trabeculae and their replacement by secondary spongiosa. (The osteoclasts could be distinguished only occasionally; see comment on "condition of specimens", above.)

Synchronous controls: the number of trabeculae seemed much like the previous group. Osteogenesis started somewhat more distally from the cartilage plate (Figure 2). Bone matrix was much more delicate than normal. Fewer of the trabeculae continued into the secondary spongiosa, leaving more showing a truncated appearance and being shorter than in the previous group.

Flight group: the number of trabeculae was somewhat reduced (with much variability in the group). Osteogenesis started still more distally from the cartilage plate, with a delicacy of bone matrix like that in the Synchronous controls. The trabeculae were much shorter than in either previous group, and only rarely continued into bone of secondary spongiosa (which was almost completely absent in this group; see below). These findings suggested excessive osteoclastic action in this group (Figure 3).

The following attempts were made to demonstrate these differences quantitatively.

(a) The length of trabeculae in the primary spongiosa was measured with an ocular micrometer (1 unit = 37 microns), from the line of erosion of the epiphyseal cartilage plate to the region where most of the bony trabeculae ceased to have cartilage cores. The average of four measurements on each specimen was used as the value for that animal.

(b) The number of trabeculae per millimeter was counted along a transverse line just below the cartilage margin.

(c) The sum of the breadths of trabeculae along that transverse baseline was determined and expressed as a percent of bone at that level, the remainder being marrow.

(d) The average breadth of trabeculae was determined from the total number and the sum of their breadths.

Results of these measurements are given in Table 1. They were interpreted as follows:

(a) The shortening of trabeculae in the primary spongiosa was clearly demonstrated. From a mean length of 817 microns in Vivarium controls, the Synchronous controls were reduced to about $2/3$ of this value (547 microns). The Flight group underwent still more reduction, to about $2/5$ normal (315 microns). There being no significant evidence of reduced growth, this shortening is more to be attributed to increased resorption and impaired bone turnover (replacement of primary by secondary spongiosa) than to failure of osteogenesis per se.

(b, c, d) Efforts to express reduction in trabecular number and increased delicacy of those remaining were not successful. An appreciable variability within groups, and the number of subjective judge-

ments used in the determinations, both contributed to the failure to give quantitative expression to these findings.

Bone: Secondary spongiosa.

Marked differences were visible among the groups in amount and extent of secondary spongiosa. In the Vivarium controls it was abundant, sturdy, and reached well into the diaphyseal marrow cavity. In Synchronous controls it was less abundant, seemed more delicate, and was of shorter extent. In the Flight group such bone was almost non-existent, the medullary bone consisting almost entirely of the short segments of primary spongiosa described above.

These findings were expressed quantitatively in part. In the medullary cavity the total depth was measured at which any medullary bone could be seen, whether sparse or abundant (Table 1). Vivarium controls had bone extending to a mean distance of 2.93 mm. beneath the cartilage. The extent in Synchronous controls was less than half of this distance (1.34 mm.). In the Flight group, where such bone was rarely to be seen, a few residual spicules could be found, at less than 1/3 the normal depth (0.93). These figures give no indication of the reduction in number of trabeculae of the secondary spongiosa; the marked difference appears in the photomicrographs (Figures 1, 2, 3).

DISCUSSION

The basic phenomena of endochondral osteogenesis, and of the bony spongiosa formed and replaced in long bones during the growth process, are summarized in histology texts and have been critically reviewed recently (1); terms and viewpoints in the following discussion are in accord with that review.

The findings described are in very substantial accord with

those reported from a previous experiment (COSMOS-605) with comparable conditions (2). The chief gains to be made from the present material are those obtainable quantitatively, either directly or by inference in comparison with data from other sources.

Some growth and maturation data on the tibia of rats are available (from normal controls in a published endocrine experiment - 3). The slight differences in source do not appear likely to foster major errors in deductions; they are summarized here.

(a) The comparison data are obtained from rats of the Long-Evans strain.

(b) Ages of the groups ranged around 3 months of age (81, 91, 101, and 111 days); explicit data on age in the present COSMOS experiment has not been received, but is understood to be approximately 3 months of age.

(c) Sex was female in the Long-Evans series, male in the present COSMOS series. Unpublished data of the author suggest that marked sexual dimorphism in tibia length is not usually seen at this age; males tend to have tibias 4 - 5% longer than females at this time, and in fact just this difference is recorded between the present COSMOS group and the data offered for comparison.

The referenced study (3) shows that this age allows an added convenience for comparison; at three months, epiphyseal-diaphyseal fusion (bony union through loss of the growth cartilage) takes place at the distal end of the tibia. Thus, the distal end has ceased to contribute to growth in bone length, and the proximal end (examined in this study) provides the total contribution to any further gains.

The Long-Evans strain comparative data indicate that during a month approximately bracketing the 3-month age, average tibial elongation is 2.4 mm.; intermediate measurements, and stability in the width of the epiphyseal cartilage plate indicate a nearly linear

rate of increase during this period. Thus, by interpolation, in a 19.5-day period (= flight time in this COSMOS study) an estimate of 1.6 - 1.7 mm. of gain in bone length appears valid. The length of spongiosa in the proximal tibial metaphysis corresponds, since formation (at the epiphyseal cartilage plate) and resorption (deeper in the diaphyseal cavity) normally keep pace in balanced growth.

Applying such information to the data in Table 1 makes it clear that a very large part of the spongiosa seen in Vivarium controls (2.93 mm. deep) would have been formed before the beginning of the experimental period, for it can be assumed (in balanced formation and resorption) that the bone found at the 1.6 - 1.7 mm. depth level at the end of the experiment was that laid down at the epiphyseal plate (or its replacement-bone) 19 days earlier. In Synchronous controls resorption must have been quite active; not only the comparable pre-existing bone, but also some of that formed during the experimental period was removed, leaving a spongy-bone depth of 1.34 mm. The marked bone resorption in the Flight group becomes still more evident then, if one recognizes that not only the (approximately) 1.2 mm. of pre-existing spongiosa, but an additional (approximately) 0.8 mm. of that which would be expected to be formed during the time in orbit was, in fact, removed; this latter is about $\frac{1}{2}$ of all of that which would have been expected to be formed during the orbiting time.

These figures cannot be absolutes, but only indications of the direction and magnitude of the spongy-bone turnover process. As such they are probably not seriously misleading. For example, with respect to actual bone growth, the following may be deduced. Even though the slight reduction in bone length in Flight animals, compared to Vivarium controls, was not statistically significant,

it is possible to examine the 0.4 mm. shortfall as a meaningful difference. The amount corresponds well with the shortfall in length of the primary spongiosa in the Flight group (502 microns, or about 0.5 mm.) lending credence to the reality of the lesser value. Yet this is a reduction of only 25% of the length gain expected during the period, suggesting that bone growth rate was at least 75% of the normal value. It is primarily at this point that the present data and those from the COSMOS-605 experiment (2) are in ambiguous accord; the present study would emphasize the defective quality (delicacy of trabeculae, and possibly mineralization inadequacy conducive to fracturing during dissection) of the bone formed, rather than any noteworthy growth retardation under hypogravity.

Finally, attaching significance to the 0.4 mm. shortfall leads to only minor alterations in the conclusion on massive spongiosa resorption in the Flight group; in addition to resorption of all spongy bone present before launching one would estimate resorption of $1/4$ (rather than $1/2$) of that formed during the flight.

SUMMARY

Tibia lengths, histological appearance of the proximal tibial epiphysis and metaphysis, and measurements of the bony spongiosa in the latter, have been reported for 6 male rats subjected to weightlessness in earth-orbit for 19.5 days, and compared with similar studies conducted on vivarium controls and on synchronous controls (experiencing similar conditions except for orbital flight).

Growth retardation was not marked, falling somewhere between 25% and a negligible level. Bone formation was slightly impaired in synchronous controls, and to an appreciably greater extent in

flight animals. Bone resorption was appreciably accelerated in synchronous controls, and still more markedly in flight animals to an extent under which virtually all pre-flight medullary bone was removed and in addition a substantial fraction ($\frac{1}{4}$ - $\frac{1}{2}$) of that formed during the flight was also resorbed.

Although the extent of change in the synchronous controls indicates that disuse atrophy and other restrictions during the experiment account for a part of the imbalance, the condition of weightlessness added a considerable further imbalance which cannot be attributed to such disuse atrophy alone.

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HISTOLOGICAL STUDIES ON TIBIAL BONE
OF RATS IN THE 1975 COSMOS-782 FLIGHT

Part 2. Microradiographic Study of Cortical Bone

by

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ABSTRACT

Based on the hypothesis that a shift in the normal balance of internal structural remodelling of bone might account for reduction of bone mineral during prolonged weightlessness in earth-orbit, a study of rat cortical bone has been made by microradiography, on samples of tibia from rats in the 1975 COSMOS-782 experiment (together with synchronous and vivarium controls). Microradiographs were examined by a micro-photodensitometer, to provide semi-quantitative measurements of bone porosity and of mineral densities ranging from minimal to maximal in four proportionate ranges. Results suggested that ranges of mineral densities in these 3-month-old vivarium control rats were in accord with findings of others on human juveniles. Synchronous controls showed increased porosity and a shift in mineral balance toward a larger bone proportion showing low mineral content. Findings from Flight animals were ambiguous, both in porosity and in a tendency toward uniformity among the various ranges of mineral content. A critique is provided, discussing possible sources of variation in the present experiment, and defining conditions which might lead to a definitive experiment.

INDEXING

Bone, internal remodelling

Cortical bone turnover

Hypogravity

This report describes studies on the dense cortical bone (tibia) of rats in the 1975 COSMOS experiment. The method of study is by microradiography of thin-ground sections of the upper diaphyseal region, and by semiquantitative evaluation of the radiographic bone densities thus recorded, to reflect possible alterations in the mineralization of dense bone. Such studies were undertaken in the hope of some relevance to the observed skeletal demineralization of astronauts under extended terms of weightlessness (1).

RATIONALE AND BACKGROUND

Microradiographs differ from conventional radiographs chiefly in the high resolution (very low graininess) of the film employed (allowing examination under compound microscope), in the thinness of specimen necessary to take advantage of such resolution, and in the x-ray beam employed (which is of low kilovoltage potential and which for beam geometry must be generated on a target face approaching a point-source). Minor differences include the small fields available and the prolonged exposures required because of low kilovoltage and low film-emulsion sensitivity (speed).

With such microradiographs it has long been recognized that dense bone (e.g., of osteonal or Haversian organization) is non-uniform in its mineralization (2). Osteones in the same field show a range varying from very poorly to very highly mineralized content, most likely reflecting the recency or longer duration of each in the life-long process of "internal remodelling" of bone under which they are constantly being resorbed, reformed, and accordingly mineralized (3). Qualitative studies of these architectural and related mineral differences have been found useful in interpreting normal age changes from youth to senescence (4) and in such disorders as osteoporosis (5).

Conceivably, if the mineral content of a bone is lowered (as in hypogravity), this could result from a shift in the balance of internal re-

modelling, in which a smaller proportion of osteones would be of the older, high mineral density nature, and a larger proportion of the recently formed and less mineralized type.

The foregoing hypothesis encounters a primary difficulty in work with rats, in that their cortical bone is not organized into osteonal architecture (except in very old males), and that the base-line for "internal remodelling" studies is less apparent. In the absence of the visual keys provided by osteonal contours (fragmentary and complete) a quantitative estimation of the varying degrees of mineral content becomes still more necessary. Furthermore, in terms of the present COSMOS experiment conditions, it is not known whether the 19.5-day flight time is a sufficiently large proportion of the mean bone turnover time to allow shifts in the remodelling balance to be detected.

Although the present report does not describe the hoped-for level of success, it has been possible to outline the conditions of a probably critical study, as subsequently described.

METHODS

The instrumentation techniques developed for this study are unfamiliar and hence described in full detail (see Appendix), later. At this point the main procedures and concepts are described.

Thin-ground transverse sections of the tibial cortex, embedded in polystyrene plastic, were microradiographed in contact with high resolution spectrographic film, under conditions of exposure and development which were rigidly standardized. The resulting films were examined microscopically; in addition to the visual ports of the microscope, one port led to a photomicrographic camera. The film carriage of the camera was replaced by a sensitive photomultiplier tube, movable in both coordinates of the focal plane (and motor-driven in the transverse axis). By use of an aperture-

diaphragm in the light pathway, and movement of the tube by motor-drive, a strip of microradiograph 6 microns wide is scanned along a 300 micron length, in a succession of photomultiplier tube outputs. Successive displacement of the scanning line allows survey of a known field area to be made (usually 75 to 100 such being made for each specimen, covering an area of the order of 0.8 - 0.9 sq. mm.).

Use of photomultiplier tube output to represent mineral densities (judged radiographically) depends on established principles of morphometry. These have been well developed on mathematical bases (6), and may be summarized as follows:

If in a block of tissue, a structural feature of interest (in this case, a given mineral-density of bone) is distributed randomly, and if a thin section of the block is prepared, its visual image may be inspected through an overlying grid.

- (a) The number of times that the feature coincides with a point (i.e. grid intersection) along one of the lines, is proportionate to the length of that portion of the test-line (as a percent) coincident with the feature of interest;
- (b) The number of times that the feature coincides with lines of the grid is proportionate to the % of area under examination in which the feature is represented;
- (c) The principle is extended to the volume of the tissue if one determines the area of special feature occupied in each of successive section-planes.

The morphometric principle was here applied, on the linear base (b) by feeding photomultiplier tube output into a servo-recorder. The pen carriage was replaced by an electrical contact which could activate any one of five electrical circuits along the length of the carriage traverse. The optical and servo-recorder combination was standardized by an A.S.A.

calibrated optical density step-wedge. Readings, converted to light transmission, were subdivided into five ranges (corresponding to the five electrical circuits) so that

- a) the least light transmission (= x-ray background darkness) was recorded from specimen areas where vascular and marrow channels produced no mineral density shadows, thus indicating "bone porosity".
- b) the remaining four light transmission bands, proportionally divided, indicated bone mineral contents of Minimal, Moderate, Marked, and Maximal ranges.

The five electrical circuits were activated, one at a time, by the servo-recorder carriage traverse and, in turn, activated one of a set of electromechanical counters. The total of counts (all five) recorded during one scan by the photomultiplier tube became the base for determining the proportion of each of the separate mineral density ranges encountered (or, of bone porosity), in accord with morphometric principle (a), above. Successive line scans satisfied (b) of these principles.

Resolving power of the system was such that while lacunae of individual osteocytes were not recognized as "porosity", all but the smallest channels in the bone specimen were thus recognized.

RESULTS

A photomicrograph of a microradiograph of a tibial cortical bone cross-section is illustrated in Figure 1; the magnification (40 X) is $\frac{1}{4}$ that used during the densitometry studies. Typically, one scan covered approximately the distance from cavity to surface.

The values obtained for the bone samples are given, by animal designation, in Table 1. (In three instances splintering at some stage of the dissections made preparation of satisfactory samples

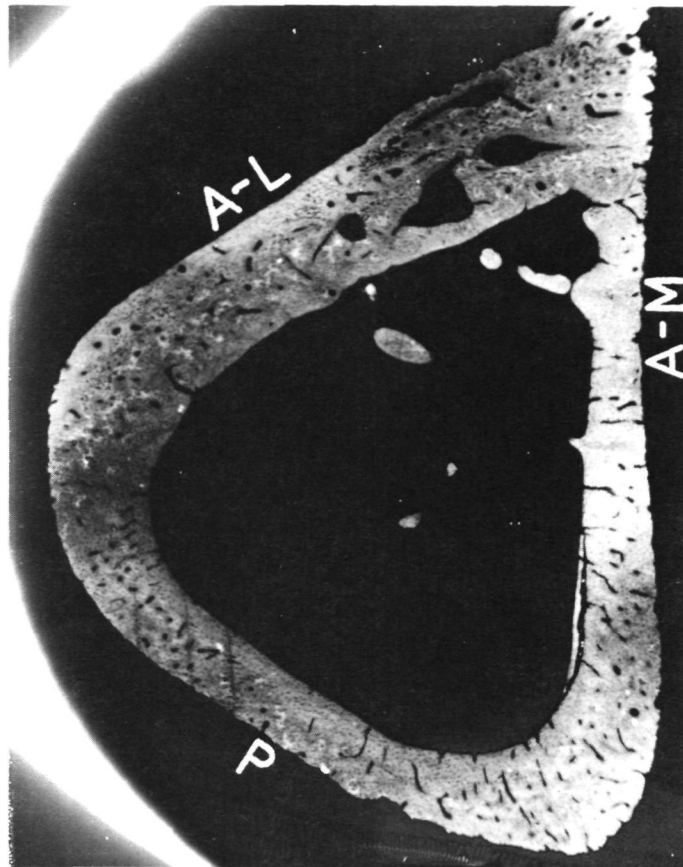


Figure 1. Microradiograph (X 40) of cortical bone in proximal metaphysis of rat tibia.

A-M: Anteromedial (subcutaneous) surface

A-L: Anterolateral (muscular) surface

P: Posterior (muscular) surface

TABLE 1

Proportions of Rat Tibial Microradiograph Area Showing (A) Porosity, and
(B) Degrees of Mineral Density.

Animal	Area Scanned, sq.mm.	Percent Porous	"Mineral Densities" as Percent of Non-porous Structure			
			Minimal	Moderate	Marked	Maximal
VO 1	0.84	5.2	12.0	45.6	26.4	16.0
VO 2	0.89	8.8	37.1	40.4	11.3	11.2
VO 3	0.89	8.7	23.8	49.0	18.1	9.1
VO 4	0.89	9.6	61.8	28.5	6.5	3.2
VO 5	0.89	17.6	58.8	35.4	6.7	0.2
VO 6	0.89	2.5	13.2	38.4	35.4	12.9
MEAN, Vivar. Cont.		8.7±2.01	27.3±7.12	39.6±2.98	37.2±9.69	8.8±2.45
SO 1	0.80	11.6	43.9	16.3	20.5	19.3
SO 2	0.77	31.6	42.3	30.9	20.2	6.6
SO 3	0.61	5.3	28.2	39.7	26.0	6.1
SO 5	0.75	29.3	99.7	0.3	-	-
SO 6	0.80	18.8	81.5	18.4	0.1	-
MEAN, Synch. Cont.		19.3±5.03	59.1±14.07	21.1±6.73	13.4±5.55	6.4±3.52
FO 2	0.84	6.1	9.7	11.7	21.9	56.7
FO 3	0.80	6.3	26.9	32.6	32.2	8.2
FO 4	0.84	6.6	13.8	30.4	33.7	22.1
FO 5	0.84	17.2	56.3	32.0	11.3	0.4
MEAN, Flight Gp.		9.1±2.72	26.7±10.53	26.7±5.01	24.8±5.20	21.9±12.45

impossible; see "Condition of specimens" in the preceding part of this report.)

The table also gives means and standard errors (corrected for small-sample size) in the three experimental groups; these means are compared by means of bar-graphs in Figure 2. It should be noted that "Porosity" represents the appropriate percent of the total area scanned. Thereafter, the four degrees of mineral density are expressed as percent of the non-porous areas (actual bone shadow). This adjustment is required to avoid false impressions of extent of mineral distribution where porosity might be markedly increased.

The bar-graph of the Vivarium controls corresponds well to that expected a priori from previous studies of others (4), in showing greater evidence for "minimal" and "moderate" mineral contents than "maximal". Juvenile bone tends to have a greater proportion of its osteones showing lower degrees of mineral content; the (approximately) three-month old rats in this study would correspond in this sense to the condition in young human beings.

At first glance, the Synchronous controls also appear to have encountered a bone-mineral imbalance which would have been expected from the histological evaluation of spongiosa in the same bones (Part 1 of this study). The larger value for porosity suggests increased resorption. The lessened proportion of bone containing Moderate to Maximal ranges of mineral suggests the greater amount of this resorption involving older bone (especially that formed before the beginning of the experimental period), and the predominance of minimally mineralized (newer?) bone is also in accord.

It is in the critical Flight group that no biological explanation can be developed to explain the findings. The lower level of

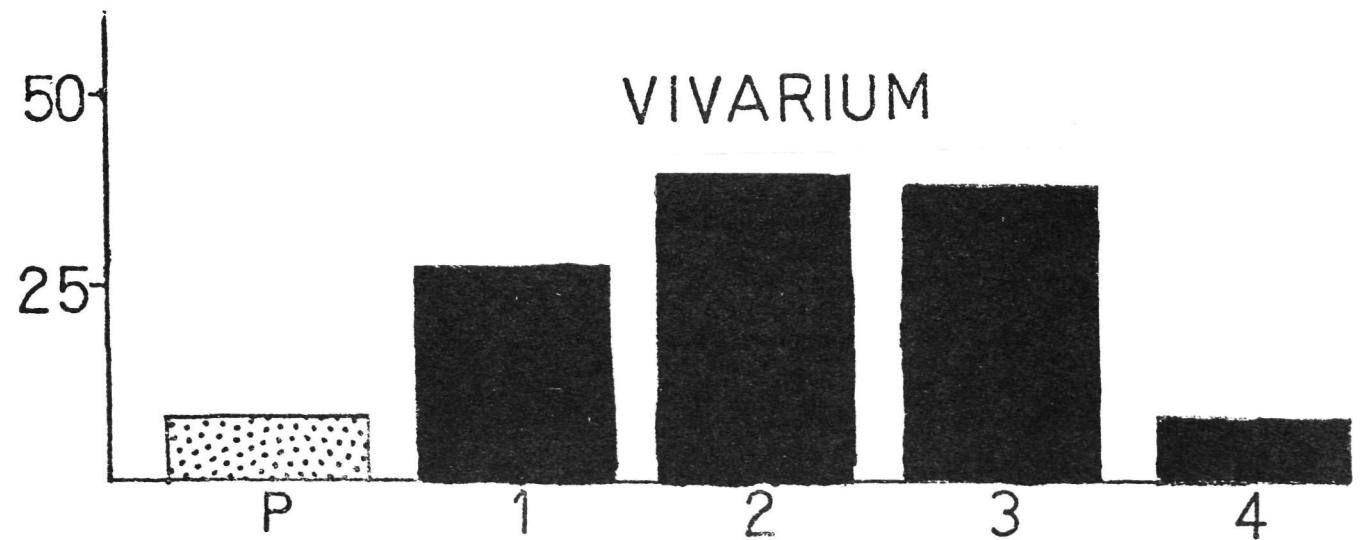
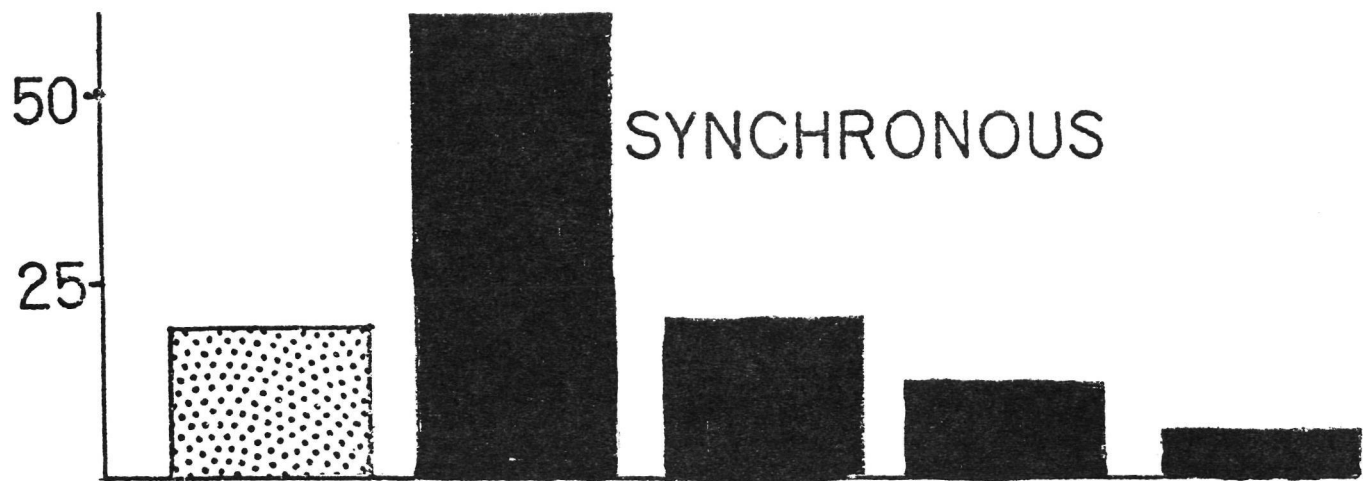


Figure 2. Graph, showing mean values from Table 1.
 P (stippled bar) = bone porosity. 1 to 4 (solid bars) representing mineral densities, in succession, as Minimal, Moderate, Marked, Maximal.

"porosity" (compared with Synchronous controls) is paradoxical, and the tendency toward a uniform distribution of mineral values is likewise difficult to interpret. As may be seen in Table 1, there was marked variability among the rats of each of the three groups, to a degree rejecting statistical significance in differences among any of the means. With only 4 specimens available for the Flight group, sampling error may have rendered the findings meaningless. It is also possible that with the prolonged period between sacrifice of animals and bone fixation, the products of marrow cytolysis may have so redistributed mineral contents of adjacent bone as to obscure any real differences which may have existed.

It can only be concluded that the present experiment is not definitive in showing that dense cortical bone (as well as medullary bone) undergoes shifts in turnover toward a lower total mineral content. However, the hypothetical basis for the experiment is not invalidated, and in place of a "Discussion" there is here offered a Critique, suggesting requirements to be established in the possible event of a similar future experiment.

CRITIQUE

1. Marked variation among individual evaluations could reflect (a) biological variability, or (b) the compounding of minor variations in the processing and analysis of samples. These latter include precision grinding to standard thickness, x-ray exposure and processing, regional and temporal variations in incident light beam in the microscope, and instrumentation in the recording system. In view of the efforts made to standardize these latter, only minor improvements are likely. The discrimination between the two sources of variability could be made by a statistically adequate study on

multiple samples from the same individuals.

2. Conceivably, the site of bone sampled could affect the outcome. For example, reference to Figure 1 shows that the tibial cross-section is roughly triangular; of these, one side is subcutaneous while the two remaining sides provide muscle attachments. If disuse atrophy is a major factor, bone alterations might be more clearly displayed adjacent to muscle attachments than on free surfaces. (In the present study, an effort was made to distribute readings approximately equally among the three surfaces.)

3. The basic concept -- that rats have some cortical bone "internal remodelling" system comparable to that in larger animals whose cortical bone has well-defined osteonal architecture -- may not be correct. Studies would therefore be required on Vivarium-maintained rats, preferably of the strain to be used in an experiment, and preferably sampling traits of juveniles, adults, and senescent groups of animals, to determine evidences of internal remodelling of rat cortical bone and to gain an impression of the length of time required to demonstrate significant structural turnover.

4. The need for optimal conditions of fixation has already been indicated.

SUMMARY

Microradiographs of cortical bone of the tibia were prepared from samples derived from groups of rats (Vivarium and Synchronous controls; Flight group) in the 1975 COSMOS-782 experiment. They were examined by a micro-photodensitometer designed to allow discrimination among radiographic shadows of vascular-marrow spaces and of mineral densities ranging from minimal to maximal in four semi-quantitative ranges. Results suggested that ranges of mineral

densities of Vivarium controls in these 3-month-old animals were in accord with findings of others on human juvenile bone. The Synchronous controls showed increased porosity and a shift in mineral balance toward a larger proportion showing low mineral content. Findings from Flight animals were ambiguous, both in porosity and in a tendency toward uniformity among the various ranges of mineral content. A critique is provided, discussing possible sources of variability in the present experiment and defining conditions which might lead to a definitive experiment.

APPENDIX

Inasmuch as parts of the experimental procedures used in this study are unfamiliar, and some of the instrumentation was specifically assembled for the study, a detailed description of these procedures is here appended.

Specimen preparation.

Cross sections of tibial cortical bone were taken at approximately $\frac{1}{4}$ of the bone's length from the proximal end, at junction of metaphysis and diaphysis. They were upgraded in successive concentrations of alcohol to 100%, transferred to acetone, and thence to a synthetic styrene monomer. After infiltration the embedding medium was polymerized with methyl-ethyl-ketone, and specimen oriented in a gelatine capsule with the polymerizing medium. After hardening sections of approximately 150 micron thickness were cut with a dental diamond-abrasive disc, and hand-ground to a uniform 40 micron thickness between two plane surfaces covered with 600-grit carborundum paper. After rinsing, the thin flakes were attached to metal washers whose aperture corresponded to the microradiographic beam. Following microradiography, bone specimens were preserved in glycerine on microscope slides.

Microradiography.

Specimens were placed in spring-loaded contact with discs of Eastman High Resolution Spectroscopic Film 649-0 (resolving power exceeding 1,000 lines/mm) and exposed in the Phillips Contact Microradiographic (CMR) apparatus. The instrument's maximum of 5 kilovolts at 2 milliamperes (10 watts) was applied for 20 minutes. (The specimen holder had been modified to give a tube-window to film distance of 16 mm., rather than the normal 9 mm. to gain a

greater field area for study, necessitating the increased exposure time.) Films were processed in developer D-19 (5 min. at 20° C.), fixed in F-1 bath, washed in running water, dipped in Photoflo, air-dried, and mounted on cardboard strips punched with an aperture suitable for study under the microscope.

Photodensitometry

A standard Leitz Ortholux photomicrographic set-up was employed, modified by use of a 6-volt ribbon filament lamp (to improve uniformity of specimen illumination). The image formed by a 6X objective and 10X periplane ocular was projected to the film-level of the camera, at 160X final magnification. In place of the film-holder a sensitive photomultiplier tube was mounted behind a 1 mm. aperture, thus allowing light from a specimen region approximately 6 microns in diameter to reach the tube. The tube and window assembly was mounted on a carriage movable in the focal plane; the lateral traverse was 48 mm. (= 300 microns of specimen image), motor driven at a constant speed; the front-to-back traverse was manually operated, and allowed as many as 5 discrete successive lateral traverses of the image to be made without moving the actual microradiograph on the microscope stage. These successive displacements were about 14 microns of actual specimen. The final number of traverses was multiplied many-fold by successive movements of the specimen with the microscope stage.

Recording and Quantitation.

The photomultiplier tube output was fed into an amplifier and servo-recorder unit. The paper-chart-graph and pen were not used; the pen carriage was modified to make any one of a series of five electrical contacts extending the full length of the pen carriage

traverse. By calibrating the microscope illumination and photomultiplier tube output with a standard A.S.A. photodensity step-wedge, five ranges of light transmission were determined (to which the system would be sensitive) and the five electrical circuits adjusted accordingly. The total range was from 1.5 to 30% transmission of incident light. The lowest subdivision of this range was adjusted to the level of x-ray background light transmission and hence, when encountered during the scanning of a bone specimen would record "empty" (vascularized or marrow) spaces in bone, reflecting porosity. The remaining four subdivisions were divided proportionately to indicate ranges defined as "minimal", "moderate", "marked", and "maximal" mineralization shadows.

(N.B.: absolute values of calcium content are not offered; under the standardized conditions of microradiography and measurement just described, the image of an aluminum foil approximately 6 microns thick yielded a light transmission of 15 percent, at the margin between "minimal" and "moderate".)

The five electrical circuits activated five electromechanical counters, which had been matched and corrected for any variations in counts/unit time. By this means, the total number of counts (sum of all five counters) in one photomultiplier tube traverse of the bone image field became 100%, and the number of counts on any one counter expressed the percent of the scan characterized by images of the defined attributes.

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MINERALIZATION IN TEETH AND JAWS,
AS JUDGED RADIOGRAPHICALLY,
IN RATS OF THE COSMOS-782 EXPERIMENT.

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INTRODUCTION

This report presents data on mineralization of the teeth in rats of the Cosmos-782 joint biology satellite experiment. Estimations were made from radiographs by optical densitometry. A previous study of effects of weightlessness in earth orbit has been reported by Soviet investigators following their cosmos-605 experiment (1). In that work the teeth were ground, the ^{45}Ca uptake was determined in vitro, and subsequently determinations of ash-organic ratio and quantitative analysis of calcium and phosphorus were made. Specific activities of ^{45}Ca were reduced by about one-half in incisor teeth immediately post-flight and continued at that level after a 25-day recovery period. The other observations, although showing differences in bones, did not establish changes in incisor teeth.

In the present study, although the pitfalls inherent in quantitative radiologic studies (2) are kept in mind, there was advantage in knowing the resorption of spongy bone in the same animals and the ambiguous results on dense bone (3). Special efforts were made to standardize the regions of tooth structure being measured, in the hope that masses of tissue of low experimental reactivity might not obscure more highly reactive sites.

Materials and Methods

There were available portions of the heads from six groups of rats, six animals per group. The groups included Vivarium controls (V), Synchronous controls (S), and Flight rats (F). Subscripts "0" or "25" indicated whether the autopsy tissues had been obtained immediately when the flight (of $19\frac{1}{2}$ day duration) terminated, or whether a 25-day recovery period was allowed before autopsy.

Heads were divided sagittally with a jeweler's saw, partially skinned and cleansed of muscles, and x-rayed on Eastman Dental X-ray Film ("Occlusal Ultra Speed D") at 14 inches (35.56 cm.) target-film distance, 65 Kilovolts, 10 milliamperes, 0.2 seconds. Development was in Eastman X-ray Developer

(manual, open tank) for 5 minutes at 20°C. A film density standard of the image of 1.1 mm. aluminum was prepared under the same conditions.

Optical densities were measured on the films with a densitometer calibrated to read in ASA (= American Standard Diffuse Density) units (Density equals $\text{Log } P_0/P_t$ where P_0 is incident radiant flux and P_t radiant flux transmitted by sample). It is recognized that radiologic estimation of mineral content is influenced not only by alterations produced by the experiment and by differences in the individual animals, but also by the variables in film composition, exposure, and processing. An attempt to hold the technique constant was therefore made, by standardizing background readings (over soft-tissue image) to 2.00, the value of the aluminum-image standard; that is, P_0 for each animal was adjusted to give a standard background. Instrumental variation was held down by restandardizing between each set of readings.

To afford maximal opportunity to detect group differences, it was necessary to select reading sites based on significant times in the experiment. This is especially necessary for incisor teeth, which undergo continuous formation, advance of position, and attrition (erosion) in rats and other rodents. Formation of the rat incisor teeth has been shown to be reasonably constant at 0.3 mm/day (maxillary tooth; the mandibular tooth's rate is slightly faster in accord with its sharpening role while maintaining occlusion⁽⁴⁾). Figure 1A illustrates an x-ray of a rat skull, and 1B is a diagram giving sites used for densitometry. The maxillary incisor tooth's curve follows the arc of a circle very closely; the radius of this arc was determined, and from that, the circumference. (The circle used followed the pulp cavity, between the outer part of the curve which is enamel and the inner part of the curve -- dentine.) That fraction of the circumference represented by any desired number of days (N) was calculated in millimeters as $0.3 \times N$, and laid out by an appropriate arc segment on

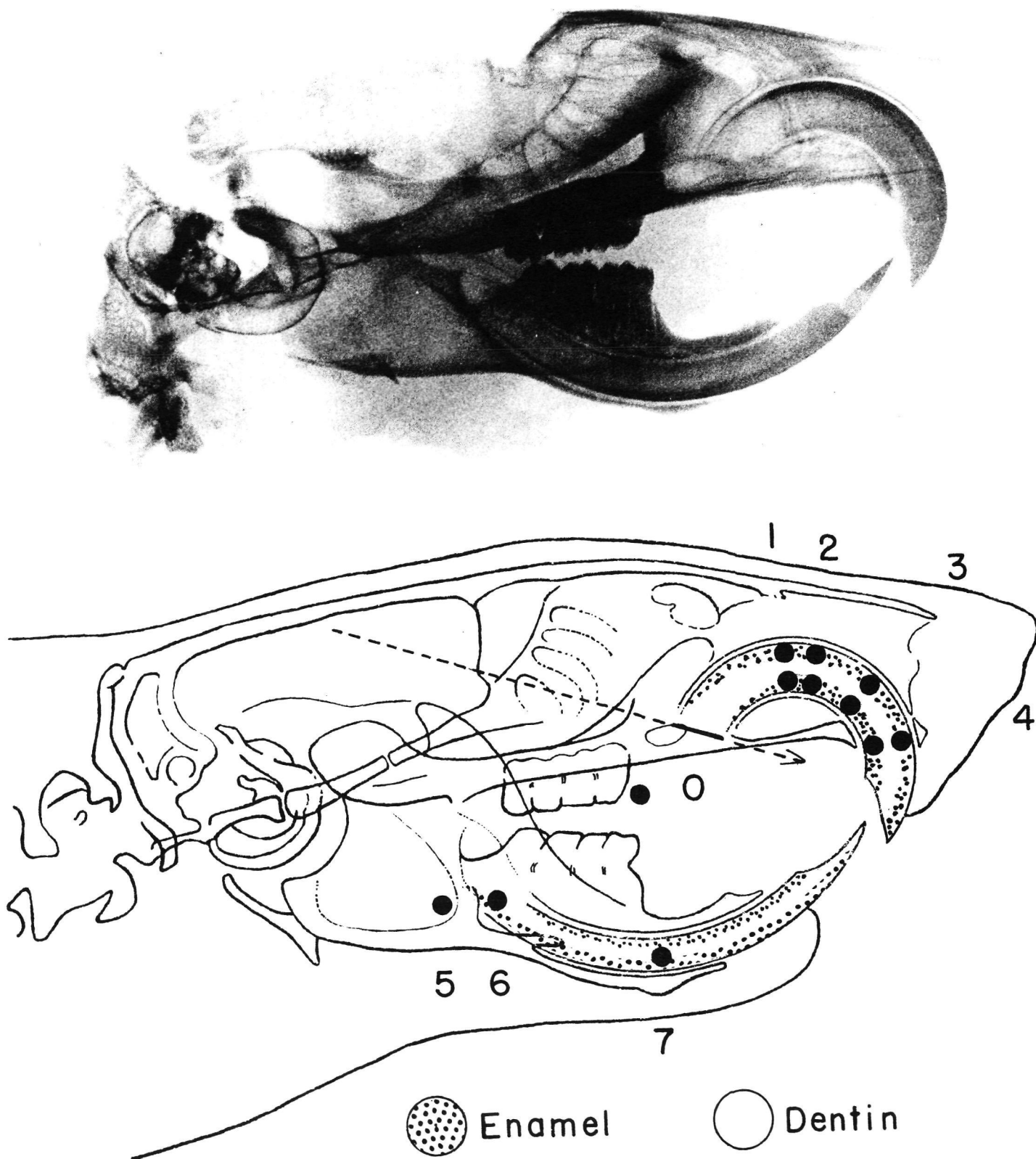


Figure 1. A: (top) X-ray (X 3.7) of left half of skull of rat V₂₅₅
 B: (bottom) Diagram showing sites used in photodensitometry of x-rays (dots to scale)

the circumference. Limitations were principally (1) that readings should be taken at some reasonable distance from the apex (region of tooth formation) to reach the mineralized band of structure, and (2) that readings should not be over the tip of the tooth (i.e., non-bony enclosure region) since the greatest part of the tooth was in fact enclosed in thin bone.

It was decided that on the F_0 series (and two associated control groups) readings should represent pre-flight status and day of vehicle launch, and that on the F_{25} (and controls) series, readings should span the actual experimental period. Both enamel and dentine should be represented, a circumstance made simple by their discrete representations, as mentioned earlier.

Table 1 represents the significance attached to the various measuring sites. It will be recognized that, because the densitometer aperture used was 1 mm. in diameter it represented a zone of approximately 3.3 days of incisor tooth formation. Thus, with small deviations, some of the indicated sites could represent different times in the two different series.

Table 1.

Definitions of Densitometry Sites Enumerated in Figure 1.

Site No.	Interpretation	
	F_0 , V_0 , S_0 Series	F_{25} , V_{25} , S_{25} Series
0	- - - - Soft Tissue Background; Standardization Site - - - -	
1	Day of Launching (D_L)	Day of Landing ($D_{+19\frac{1}{2}}$)
2	7 days before Launch (D_{-7})	not used
3	not used	Mid-flight (D_{+10})
4	28 days before Launch (D_{-28})	Day of Launching (D_L)
5	- - - - - Thin bone, body of mandible - - - - -	
6	- - - - - Heavy " " " " - - - - -	
7	- Composite (enamel, dentine, pulp, trace of alveolar bone)	

Because the actual mineralization represented will be a reciprocal of the optical densities, it is clear that the darker a region of x-ray film, the higher is the density reading but the lower the mineral in the structure.

To allow more easily interpreted graphic representation, mean values of optical densities were converted to the light transmission (as % of incident light) for each structure in each group, thereby permitting increasing mineralization to be represented by higher numerical values. A standard conversion table is shown as Appendix A. It will be noted that while the density readings follow a logarithmic scale, the light transmission values are on an arithmetic percentage scale.

Results

Table 2 gives means and standard errors of the optical densities in the various experimental groups and at the various sites. ("Student's" correction for the small sample group size was used.) The same data, converted into percent transmission, appears in graphs as Figures 2 and 3.

In almost every instance, individual variation within groups was rather high; in this respect the findings are like the radiographic analyses obtained from human subjects' skeletons in the Gemini and early Apollo missions mentioned earlier (2). Statistically significant differences ($p \leq 0.01$ or lower) were often not detectable with such variation. (Coefficients of variation, V , are not given but are easily obtained by reconstructing the standard deviation -- i.e., multiplying standard error by the square root of the number of cases [$\sqrt{6} = \text{approx. } 2.45$], and applying the formula $V = 100 \times \text{standard deviation} \div \text{the mean}$.) With such variation, differences of 25% or more are often required to demonstrate statistical significance.

1. Values in enamel are quite stable in every group (Figure 2, top). They showed no significant change over time. Although enamel values in F groups (both F_0 and F_{25}) seemed lower than control levels, the difference was not significant. These findings seem reasonable in view of the large crystal size and general stability of enamel hydroxyapatite.

Table 2

Optical Density (ASA Standard at Sites shown in Figure 1B)
(Means & Standard Errors, 6 rats/Group)

Group	INCISOR ENAMEL				INCISOR DENTINE				THIN BONE	HEAVY BONE	COMPOSITE
	1	2	3	4	1	2	3	4	5	6	7
	D _L Day of Launch	D ₋₇ 1 week pre-Launch	-	D ₋₂₈ 4 weeks pre-Launch	D _L Day of Launch	D ₋₇ 1 week pre-Launch	-	D ₋₂₈ 4 weeks pre-Launch	- - Day of Landing - -		
314 V ₀ Vivarium Controls	1.69 ± .029	1.67 ± .031		1.71 ± .021	1.85 ± .019	1.81 ± .019		1.76 ± .022	1.68 ± .033	1.56 ± .043	1.68 ± .033
F ₀ Flight Group	1.76 ± .028	1.74 ± .013		1.73 ± .022	1.92 ± .016	1.88 ± .020		1.81 ± .029	1.73 ± .024	1.63 ± .025	1.73 ± .027
S ₀ * Synchron. Controls *5 rats in group	1.66 ± .076	1.65 ± .067		1.65 ± .066	1.83 ± .049	1.79 ± .030		1.72 ± .057	1.76 ± .016	1.63 ± .028	1.61 ± .073
	D 19½ Day of Landing	-	D 10 Mid- Flight	D _L Day of Launch	D 19½ Day of Landing	-	D 10 Mid- Flight	D _L Day of Launch	25 Days after Landing		
V ₂₅ Vivarium Controls	1.67 ± .066	1.65 -	1.65 ± .034	1.68 ± .063	1.85 ± .048		1.77 ± .056	1.74 ± .049	1.72 ± .033	1.51 ± .019	1.65 ± .061
F ₂₅ Flight Group	1.72 ± .015		1.76 ± .014	1.78 ± .033	1.97 ± .018		1.84 ± .010	1.80 ± .033	1.65 ± .038	1.49 ± .031	1.66 ± .041
S ₂₅ Synchron. Controls	1.67 ± .032		1.66 ± .021	1.69 ± .024	1.88 ± .035		1.78 ± .032	1.76 ± .027	1.65 ± .021	1.50 ± .016	1.66 ± .031

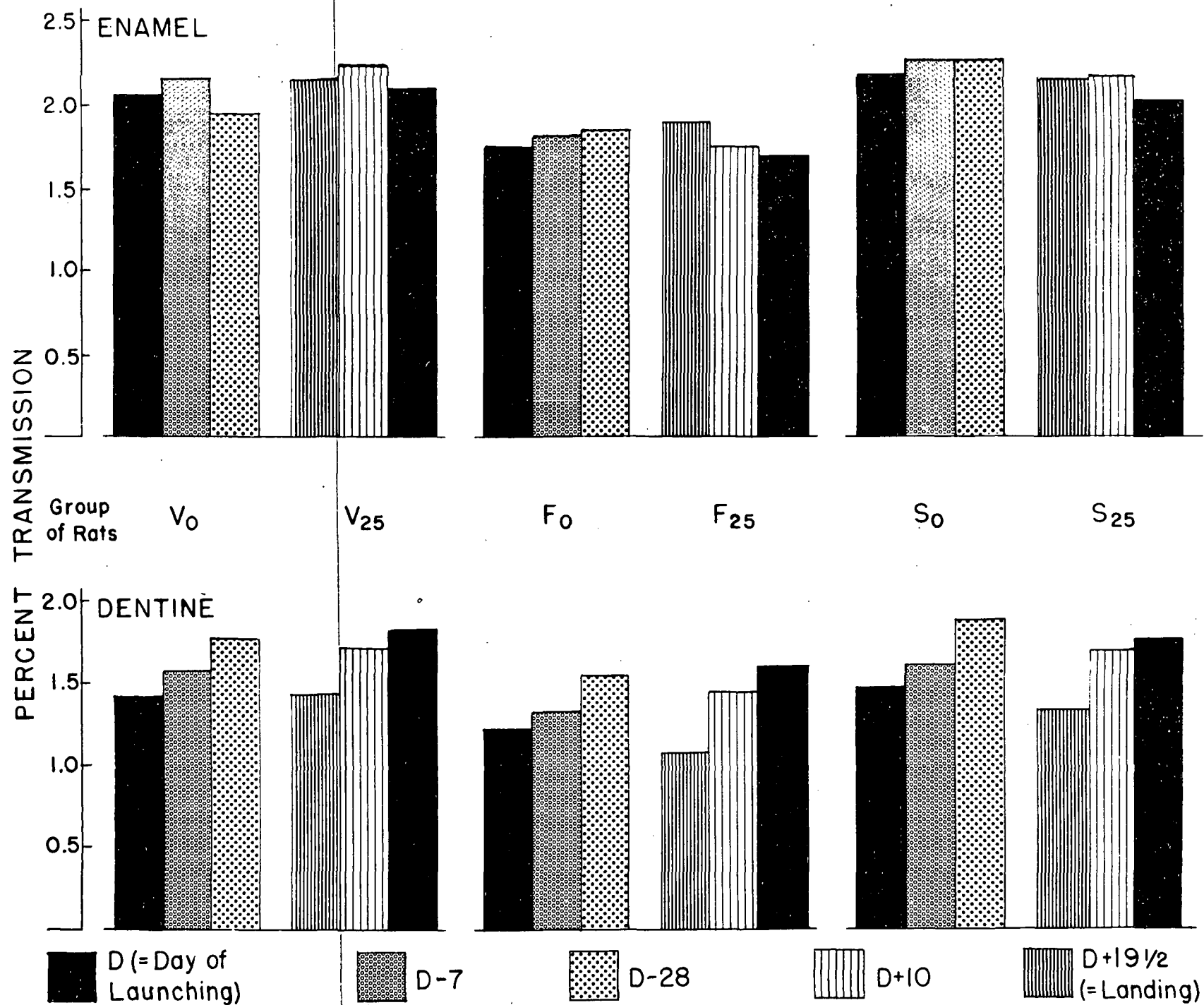


Figure 2

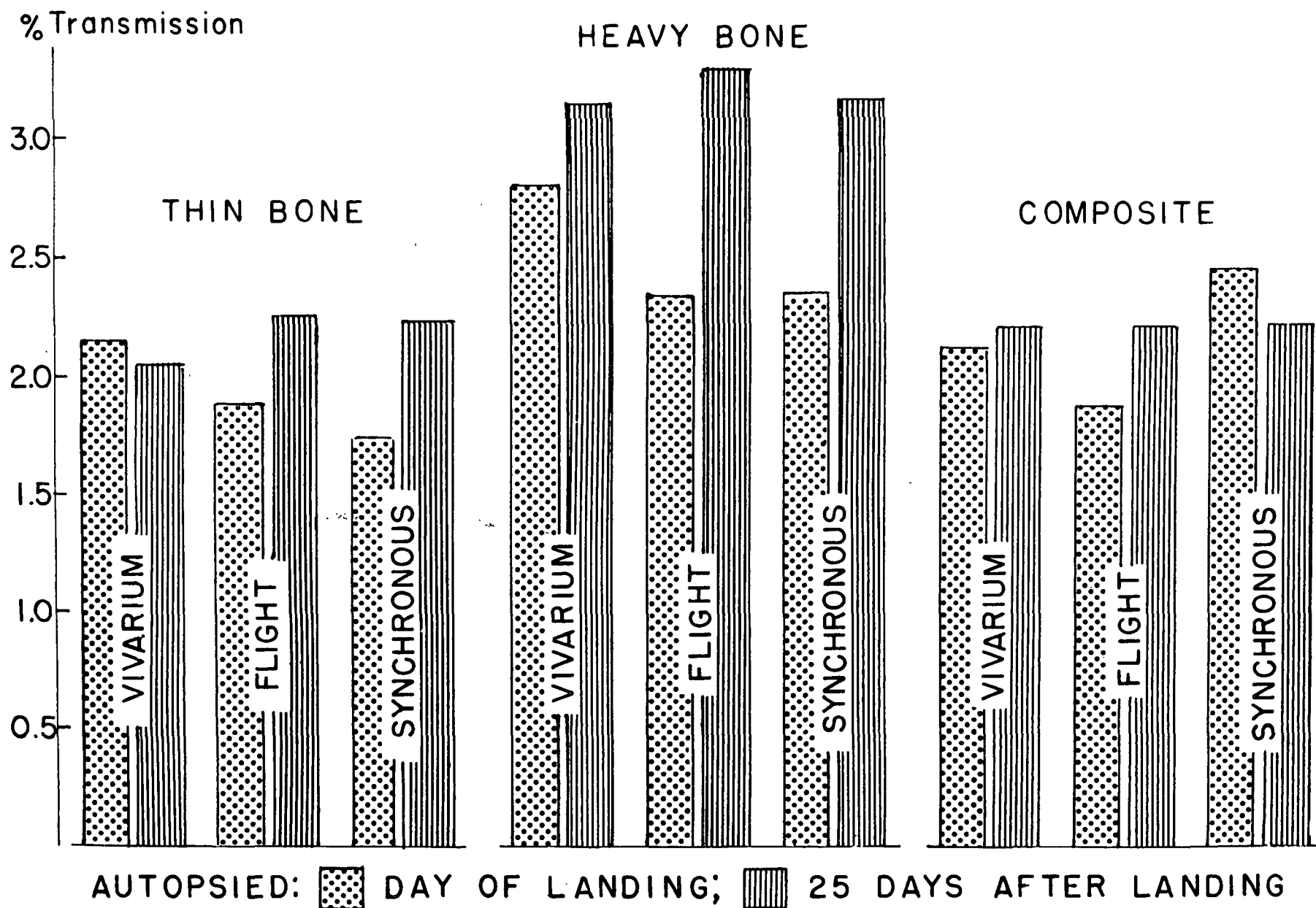


Figure 3

2. In every group, dentine values rose as observation passed from regions of more recently formed to earlier-formed dentine (bottom, Fig. 2). In every instance the gain was significant between the extreme values (28 days apart in F_0 series and controls, $19\frac{1}{2}$ days apart in the F_{25} and related groups), except in the S_{25} group, where $p = 0.05$. Readings at intermediate levels were not significantly changed. Synchronous and vivarium control values were alike; the F_0 and F_{25} values appeared lower and were possibly significantly so ($p = 0.05$) except for the oldest dentine in each group, where mineral did not differ significantly from controls. These findings suggest

- a) that dentinal mineralization increases progressively during the formation period of the tooth; and
- b) that a small amount of mineral loss (without any tooth resorption detectable in corresponding histological sections) may have occurred in flight animals during the experimental period; and
- c) that mineral repletion was not observed during the recovery period.

3. Dentinal mineral content was initially substantially below that of enamel at the same developmental level of tooth (p 0.01 to 0.001 in the newest and the intermediate age tooth structure), but with the rising content in the dentine it reaches a value not significantly less than that of the enamel. Flight animals were like control groups in this respect.

4. Figure 3 shows that thin bone of the mandibular body was slightly reduced below Vivarium control level in both F_0 and S_0 rats. During the 25-day recovery period it rose to Vivarium control levels in both groups. In each instance significance of difference was in some doubt $--> 0.05$ in F groups, and $= 0.05$ in S groups. Changes of similar direction were found in the heavy bone underlying the molar teeth. Although the reduction from initial Vivarium control level was not significant, the gain in both F_{25}

and S25 rats was clearly significant. The reason for these bone changes is not immediately obvious. Unlike the loss of spongiosa in the tibia they were seen in similar degree in both Flight and Synchronous control groups in the laminar bone of the mandible. It is possible that if the nature of the diet furnished to these two groups was markedly softer than in the Vivarium control group ration, the stimulus of chewing would have been much reduced in F and S rats; restoration to a firmer diet during the 25-day recovery period might then have increased masticatory activity and improved bone structure. The conceptual basis for mechanical stimulation of bone formation and resorption has been well established, and recently reviewed(5)

5. The "composite" reading in the region of the mandibular incisor was taken in order to obtain a mixed sample of dental tissues at about the mid-point of the formation period; it was hoped possibly to simulate the composite of tissue samples studied chemically in the pulverized incisor tooth, as had been done in the Soviet study previously mentioned(1) The densitometry readings allowed no conclusions as to consistent trends to be drawn, and in this respect were similar to the ash assays in the earlier study.

APPENDIX A

CONVERSION TABLE

Optical Density vs. Percent Transmission

Density Percent Transmission or Reflection	Density Percent Transmission or Reflection	Density Percent Transmission or Reflection	Density Percent Transmission or Reflection	Density Percent Transmission or Reflection	Density Percent Transmission or Reflection	Density Percent Transmission or Reflection	Density Percent Transmission or Reflection
0.00 100.00	0.50 31.62	1.00 10.00	1.50 3.162	2.00 1.000	2.50 0.3162	3.00 0.1000	3.50 0.0316
0.01 97.72	0.51 30.90	1.01 9.772	1.51 3.096	2.01 0.9772	2.51 0.3090	3.01 0.0977	3.51 0.0309
0.02 95.50	0.52 30.20	1.02 9.550	1.52 3.020	2.02 0.9550	2.52 0.3020	3.02 0.0955	3.52 0.0302
0.03 93.33	0.53 29.51	1.03 9.333	1.53 2.951	2.03 0.9333	2.53 0.2951	3.03 0.0933	3.53 0.0295
0.04 91.20	0.54 28.84	1.04 9.120	1.54 2.884	2.04 0.9120	2.54 0.2884	3.04 0.0912	3.54 0.0288
0.05 89.13	0.55 28.18	1.05 8.913	1.55 2.818	2.05 0.8913	2.55 0.2818	3.05 0.0891	3.55 0.0289
0.06 87.10	0.56 27.54	1.06 8.710	1.56 2.754	2.06 0.8710	2.56 0.2754	3.06 0.0871	3.56 0.0275
0.07 85.11	0.57 26.92	1.07 8.511	1.57 2.692	2.07 0.8511	2.57 0.2692	3.07 0.0851	3.57 0.0269
0.08 83.18	0.58 26.30	1.08 8.318	1.58 2.630	2.08 0.8318	2.58 0.2630	3.08 0.0832	3.58 0.0263
0.09 81.28	0.59 25.70	1.09 8.128	1.59 2.570	2.09 0.8128	2.59 0.2570	3.09 0.0813	3.59 0.0257
0.10 79.43	0.60 25.12	1.10 7.943	1.60 2.512	2.10 0.7943	2.60 0.2512	3.10 0.0794	3.60 0.0251
0.11 77.62	0.61 24.55	1.11 7.762	1.61 2.455	2.11 0.7762	2.61 0.2455	3.11 0.0776	3.61 0.0245
0.12 75.86	0.62 23.99	1.12 7.586	1.62 2.399	2.12 0.7586	2.62 0.2399	3.12 0.0759	3.62 0.0239
0.13 74.13	0.63 23.44	1.13 7.413	1.63 2.344	2.13 0.7413	2.63 0.2344	3.13 0.0741	3.63 0.0234
0.14 72.44	0.64 22.91	1.14 7.244	1.64 2.291	2.14 0.7244	2.64 0.2291	3.14 0.0724	3.64 0.0229
0.15 70.79	0.65 22.39	1.15 7.079	1.65 2.239	2.15 0.7079	2.65 0.2239	3.15 0.0708	3.65 0.0223
0.16 69.18	0.66 21.88	1.16 6.918	1.66 2.188	2.16 0.6918	2.66 0.2188	3.16 0.0692	3.66 0.0218
0.17 67.61	0.67 21.38	1.17 6.761	1.67 2.138	2.17 0.6761	2.67 0.2138	3.17 0.0676	3.67 0.0214
0.18 66.07	0.68 20.89	1.18 6.607	1.68 2.089	2.18 0.6607	2.68 0.2089	3.18 0.0661	3.68 0.0218
0.19 64.57	0.69 20.42	1.19 6.457	1.69 2.042	2.19 0.6457	2.69 0.2042	3.19 0.0646	3.69 0.0204
0.20 63.10	0.70 19.95	1.20 6.310	1.70 1.995	2.20 0.6310	2.70 0.1995	3.20 0.06310	3.70 0.0199
0.21 61.66	0.71 19.50	1.21 6.166	1.71 1.950	2.21 0.6166	2.71 0.1950	3.21 0.0617	3.71 0.0195
0.22 60.26	0.72 19.05	1.22 6.026	1.72 1.905	2.22 0.6026	2.72 0.1905	3.22 0.0603	3.72 0.0190
0.23 58.88	0.73 18.62	1.23 5.888	1.73 1.862	2.23 0.5888	2.73 0.1862	3.23 0.0588	3.73 0.0186
0.24 57.54	0.74 18.20	1.24 5.754	1.74 1.820	2.24 0.5754	2.74 0.1820	3.24 0.0575	3.74 0.0182
0.25 56.23	0.75 17.78	1.25 5.623	1.75 1.778	2.25 0.5623	2.75 0.1778	3.25 0.0562	3.75 0.0178
0.26 54.95	0.76 17.38	1.26 5.495	1.76 1.738	2.26 0.5495	2.76 0.1738	3.26 0.0550	3.76 0.0174
0.27 53.70	0.77 16.98	1.27 5.370	1.77 1.698	2.27 0.5370	2.77 0.1698	3.27 0.0537	3.77 0.0169
0.28 52.48	0.78 16.60	1.28 5.248	1.78 1.660	2.28 0.5248	2.78 0.1660	3.28 0.0529	3.78 0.0166
0.29 51.29	0.79 16.22	1.29 5.129	1.79 1.622	2.29 0.5129	2.79 0.1622	3.29 0.0513	3.79 0.0162
0.30 50.12	0.80 15.85	1.30 5.012	1.80 1.585	2.30 0.5012	2.80 0.1585	3.30 0.0501	3.80 0.0158
0.31 48.98	0.81 15.49	1.31 4.898	1.81 1.549	2.31 0.4898	2.81 0.1549	3.31 0.0489	3.81 0.0155
0.32 47.86	0.82 15.14	1.32 4.786	1.82 1.514	2.32 0.4786	2.82 0.1514	3.32 0.0478	3.82 0.0152
0.33 46.77	0.83 14.79	1.33 4.677	1.83 1.479	2.33 0.4677	2.83 0.1479	3.33 0.0468	3.83 0.0148
0.34 45.71	0.84 14.45	1.34 4.571	1.84 1.445	2.34 0.4571	2.84 0.1445	3.34 0.0457	3.84 0.0145
0.35 44.67	0.85 14.13	1.35 4.467	1.85 1.413	2.35 0.4467	2.85 0.1413	3.35 0.0446	3.85 0.0141
0.36 43.65	0.86 13.80	1.36 4.365	1.86 1.380	2.36 0.4365	2.86 0.1380	3.36 0.0436	3.86 0.0138
0.37 42.66	0.87 13.49	1.37 4.266	1.87 1.349	2.37 0.4266	2.87 0.1349	3.37 0.04266	3.87 0.0135
0.38 41.69	0.88 13.18	1.38 4.169	1.88 1.318	2.38 0.4169	2.88 0.1318	3.38 0.0417	3.88 0.0132
0.39 40.74	0.89 12.88	1.39 4.074	1.89 1.288	2.39 0.4074	2.89 0.1288	3.39 0.0407	3.89 0.0129
0.40 39.81	0.90 12.59	1.40 3.981	1.90 1.259	2.40 0.3981	2.90 0.1259	3.40 0.0398	3.90 0.0126
0.41 38.90	0.91 12.30	1.41 3.890	1.91 1.230	2.41 0.3890	2.91 0.1230	3.41 0.0389	3.91 0.0123
0.42 38.02	0.92 12.02	1.42 3.802	1.92 1.202	2.42 0.3802	2.92 0.1202	3.42 0.0380	3.92 0.0120
0.43 37.15	0.93 11.75	1.43 3.715	1.93 1.175	2.43 0.3715	2.93 0.1175	3.43 0.0371	3.93 0.0117
0.44 36.31	0.94 11.48	1.44 3.631	1.94 1.148	2.44 0.3631	2.94 0.1148	3.44 0.0363	3.94 0.0114
0.45 35.48	0.95 11.22	1.45 3.548	1.95 1.122	2.45 0.3548	2.95 0.1122	3.45 0.0355	3.95 0.0112
0.46 34.67	0.96 10.96	1.46 3.467	1.96 1.096	2.46 0.3467	2.96 0.1096	3.46 0.0347	3.96 0.0109
0.47 33.88	0.97 10.72	1.47 3.388	1.97 1.072	2.47 0.3388	2.97 0.1072	3.47 0.0339	3.97 0.0107
0.48 33.11	0.98 10.47	1.48 3.311	1.98 1.047	2.48 0.3311	2.98 0.1047	3.48 0.0331	3.98 0.0105
0.49 32.36	0.99 10.23	1.49 3.236	1.99 1.023	2.49 0.3236	2.99 0.1023	3.49 0.0324	3.99 0.0102
0.50 31.62	1.00 10.00	1.50 3.162	2.00 1.000	2.50 0.3162	3.00 0.1000	3.50 0.0316	4.00 0.0100

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QUANTITATIVE ANALYSIS OF SELECTED BONE PARAMETERS

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ABSTRACT

The effect of space flight on bone formation and mineralization, bone resorption, bone length, bone density and pore size distribution, and bone mechanical properties in rats was investigated and compared to vivarium and synchronous controls.

The most striking effects were those on bone formation; all parameters investigated in the flight animals immediately after flight were significantly decreased from both the vivarium and synchronous control groups. An arrest line was found at both the endosteum and the periosteum of the flight animals suggesting that a complete cessation of bone growth occurred during space flight. By 25 days postflight, the flight animals showed a significant increase in bone formation when compared to the vivarium controls suggesting that a rebound in bone formation occurred following flight.

INTRODUCTION

Microscopic examination of the femur, tibia, and humerus of Wistar rats following a 22 day space flight aboard Cosmos 605 showed decreased metaphyseal bone which was usually combined with a decrease in primary spongiosa mass in the vicinity of the epiphyseal cartilaginous plate suggesting an inhibition of bone growth during flight (1). Wide osteocyte lacunae were noticed and were attributed to perilacunar osteolysis. Most, but not all, of these parameters had returned to normal by 27 days after flight. Although the mineral content of bone in the flight rats did not show any significant difference, the specific activity of ^{45}Ca , in vitro, decreased about 40% again suggesting a decrease in bone formation (Prokhonchukov et al., personal communications). Bone density determinations of the os calcis and distal radius and ulna in the Skylab astronauts also suggested that a loss of bone mineral in the weight bearing bones occurred; this loss became more prevalent with an increase in the duration of flight (2).

If bones are formed in relation to gravitational stresses (3), one would anticipate that prolonged recumbency and/or prolonged weightlessness would be associated with hypercalciuria, bone demineralization, and, ultimately, osteoporosis (4, 5). The extent and duration of such changes may be an important factor in determining the necessity of a gravitational field during long term space flight. To better understand the effect of spaceflight on bone, parameters including bone formation and mineralization, bone resorption, bone length, bone density and pore size distribution, and bone mechanical properties were studied in rats both immediately and 25 days after a 19.5 day flight aboard Cosmos 782.

METHODS

PROTOCOL

Specific pathogen free, male, Wistar rats from the Institute of Endocrinology of the Czechoslovak Academy of Science were used. The animals were 63 days of age and weighed an average of 215 grams at the beginning of the experimental period. All rats were placed on the flight diet 2 weeks prior to experimentation and were given a ration of 40 gram/rat/day of the diet while water was given ad libitum. The lighting schedule was 12 hours light: 12 hours dark.

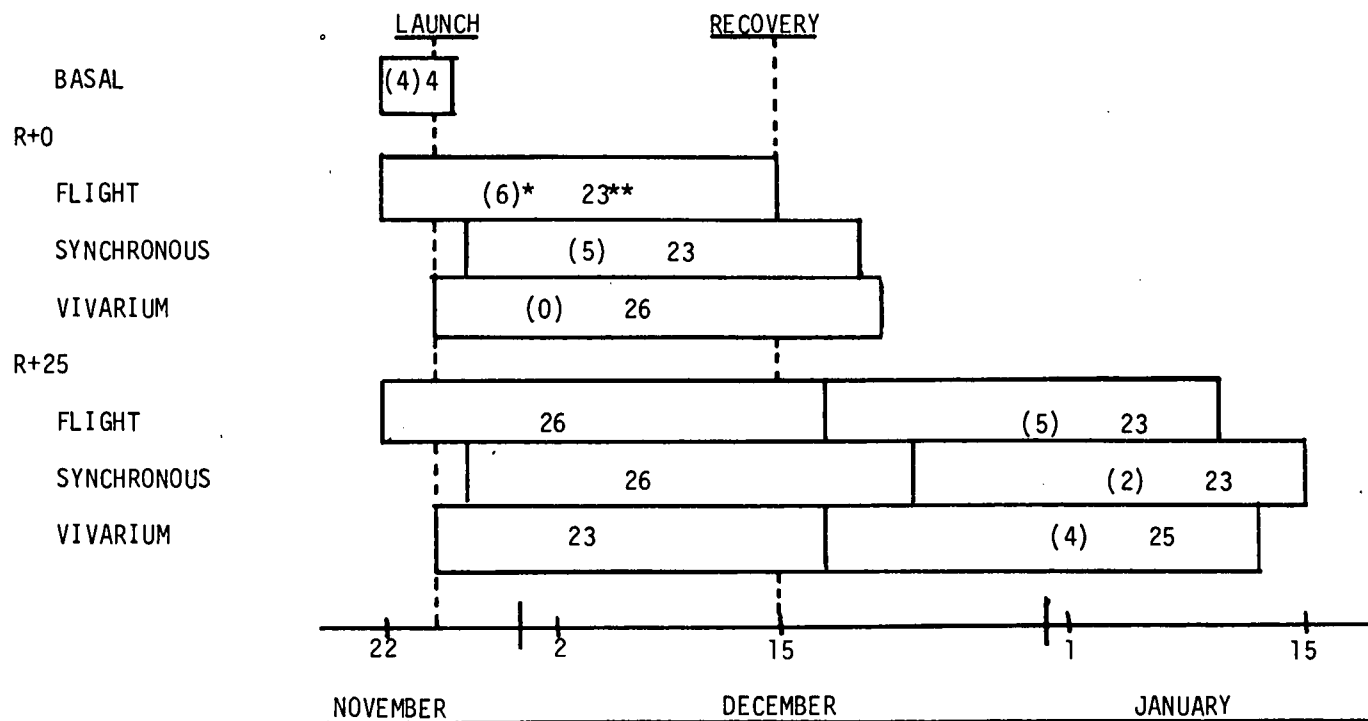
The flight rats were loaded into individual cylindrical cages (20 cm X 10 cm diameter) 20 hours prior to launch. In this hardware configuration, five cages shared common water and food sources. The synchronous controls were placed in a caging system identical to the flight group and were exposed, as nearly as possible, to the dynamic stresses and the spacecraft environment of the flight group. The vivarium controls were kept 3-4/cage. During the recovery phase, the flight and synchronous rats were kept in the vivarium in individual cages; the flight animals received an additional 5 grams of food a day.

The experimental protocol is illustrated in Fig. 1. All groups were injected with tetracycline¹, 1 mg/kg, intraperitoneally, 3 days prior to the beginning of the experimental period. A basal group was sacrificed shortly after launch. A second tetracycline injection was to be given at the time of sacrifice of the R+0 group. However, due to unforeseen weather problems in the recovery area, this injection was delayed for 3 days in all but the vivarium controls. Two different dates were given for the second tetracycline injection of the vivarium controls, Dec. 18

¹ Declomycin (demethylchlortetracycline) courtesy of Mr. John Hill of Lederle Laboratories, Pearl River, NY.

FIGURE 1

SCHEDULE OF EVENTS



* Number in parenthesis is the number of animals in each group which received the tetracycline label

** Number not in parenthesis is the length in days of each tetracycline label

and Dec. 23, with the former date verified as the valid date; this change strongly impacts the results and indicates that the R+25 vivarium controls were the only group that received a second tetracycline injection prior to sacrifice of the R+0 controls.

At the end of the experimental period, the rats were killed and the tibias and left humerus removed and immediately frozen in liquid nitrogen. The bones were kept frozen until analysed. The left humerus was used for measurement of bone strength parameters and bone density and pore size distribution. The left tibia was used for measurement of bone formation, mineralization and resorption rates while the right tibia was used for cell counts.

Because a detailed treatment of the methods used in this study has been given elsewhere (6, 7, 8, 9), only a brief description of the parameters measured will be presented.

BONE PARAMETERS

1) Bone density and pore size distribution were measured by mercury porosimetry (6). For these measurements, the pieces of diaphyseal bone left after fracture were ashed to remove cellular material from the pores. The ashed sample was then placed in the sample chamber of the porosimeter ~~which was evacuated to less than 10 millitorr~~, backfilled with mercury to atmospheric pressure, and placed in the pressure chamber which had been filled with isopropanol. As the pressure was increased, the volume of mercury intruded into the sample was determined from the level of mercury in the graduated penetrometer stem. Pressure was held constant at each point for a sufficient time so that the mercury ceased flowing into the sample. Since the pressure required to force mercury into a given pore is inversely proportional to the pore diameter, pore size distribution in the sample can be calculated. Bone density was measured by trichloroethylene

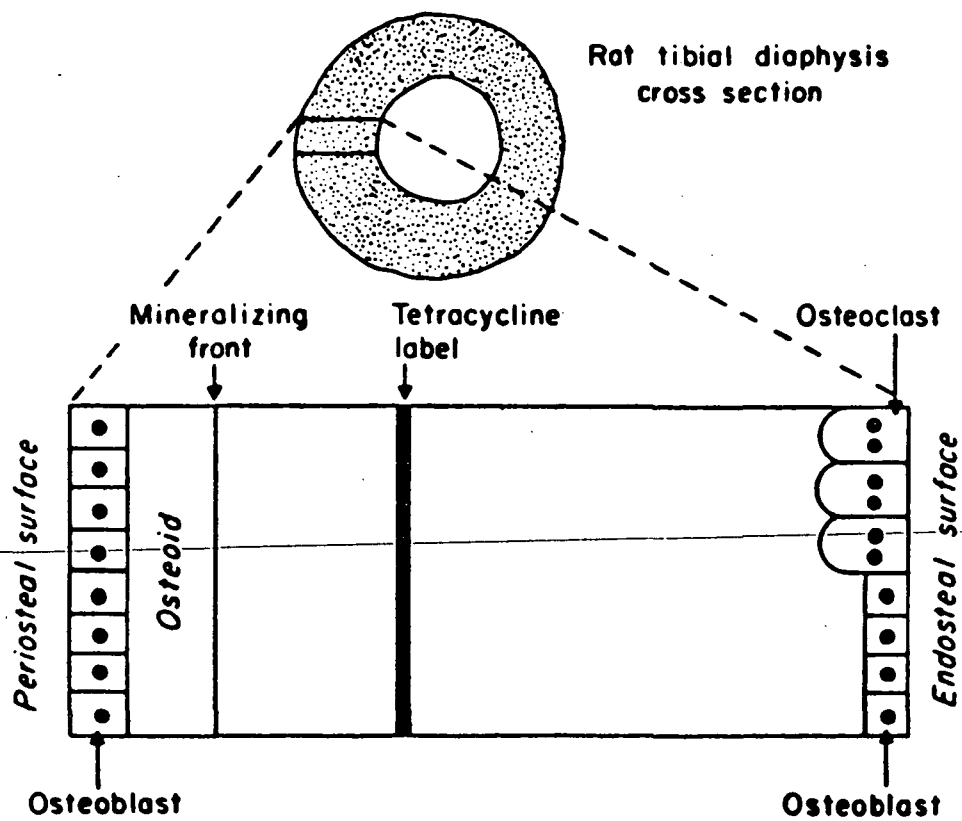
displacement in a volumetric pycnometer after vacuum (5 millitorr) infiltration to avoid entrapping air in the pores.

2) Bone mechanical parameters were evaluated with the standard torsion test machine designed by Burstein and Frankel (7). The humerus was maintained in a moistened condition at all times. The ends of the humerus were embedded in an epoxy resin prior to testing. Data was displayed on a storage oscilloscope. The torque-angular displacement curve which resulted after specimen failure was reduced to obtain ultimate torque, deformation to failure, strain energy, and stiffness.

3) Bone formation, mineralization, and resorption rates were determined by quantitative histological techniques using the left tibia while the right tibia was used for counting osteoblastic and osteoclastic cell populations. Each tibia was divided at right angles to its long axis at the fibular junction and two consecutive transverse sections, about 50 μ thick, were sawed from the proximal segment using a Gillings-Hamco thin-sectioning machine. The bone specimen holder was mounted on a goniometer to facilitate sawing sections which were perpendicular to the long axis of the diaphysis. The sawed sections were then hand-ground to a final thickness of about 30 μ . All of the variables measured except the width of the in vitro tetracycline label are illustrated schematically in Fig. 2. One left ground section was mounted unstained in Abopon (Valnor Corp., Brooklyn, NY), a water-soluble mounting medium, and used for measurements of area, width, and surface length. These measurements were performed using a model 1010A Grafacon tablet (Compunetics Inc., Monroeville, PA) interfaced with a computer (PDP-8, Digital Equipment Co., Manor, MS). In principle, the method used to measure bone formation and resorption relies on the fact that in the tibial diaphysis the periosteal circumference

FIGURE 2

FEATURES OF THE BONE SAMPLE



enlarges by bone formation and the endosteal circumference enlarges by resorption. Tetracycline is given to mark all periosteal bone formed during the experimental period. Bone resorption is determined by the change in medullary area and by measurement of total endosteal formation during the experimental period. Since endosteal formation is intermittent, a continuous tetracycline label is necessary to quantify this parameter. In this study, only measurement of the medullary area was possible. The more proximal ground section was stained with a modified von Kossa procedure, counterstained with nuclear fast red, and mounted in Fluormount (E. Gurr Ltd., London, S. W. 14, England) for measurement of osteoid width. Because the methods used to collect and analyze data have been described in detail (8, 9), only certain modifications and a description of the parameters relating to the processes of formation, mineralization, and resorption are described below.

A) Periosteal bone formation rate (mm^3/day). This is the amount of mineralized bone formed at the periosteum per day. Since tetracycline deposits in the mineralization front of bone formed before the start of the measuring period, an in vitro label was used to correct for background diffusion. The in vitro label is subtracted from the total amount of periosteal bone between labels or between the tetracycline label and the periosteum. The total bone formation rate is the sum of the periosteal and endosteal bone formation rates. The latter parameter and the bone formation which occurs around vascular canals and which makes up about 10% of the bone formation occurring in this sampling site (8) were not measured in this study.

B) Periosteal matrix formation rate (mm^3/day). This includes all periosteal matrix formed, regardless of whether it mineralizes during the

the measuring period. It is usually identical to the periosteal bone formation rate except when there is a mineralization defect such that the amount of osteoid formed is greater than the amount mineralized during the measuring period.

C) Periosteal bone apposition rate (μ /day). This is the width of periosteal mineralized bone added per day and is calculated by dividing the area of periosteal bone formed by the length of the periosteal forming surface. Apposition is also termed linear formation rate.

D) Periosteal matrix apposition rate (μ /day). This is the width of new periosteal matrix added per day and includes both osteoid and mineralized matrix.

E) Periosteal osteoid maturation rate (%/hr). This is a measure of the onset of mineralization. A certain amount of time elapses between the deposition of osteoid and the initiation of mineralization in this osteoid. This time can be calculated by dividing the width of osteoid by the matrix apposition rate. If one assumes that osteoid is 0% mature when it is formed and 100% mature when mineralization is initiated, the above time is the time to reach 100% maturity. Therefore, the osteoid maturation rate is calculated simply by dividing 100% by the above time.

This calculation makes no assumptions about the nature of the changes which must occur before mineralization can be initiated. The term "maturation" is used because a chemical change does occur in osteoid prior to the onset of mineralization (10).

A previous study demonstrating a direct relationship between osteoid width and matrix apposition (9) suggests that osteoid maturation rate is probably a better measurement of the onset of mineralization than is osteoid width. Accordingly, an increase in osteoid width could be related

to either an increase in apposition rate or a delay in the onset of mineralization, whereas the osteoid maturation rate is independent of apposition.

F) Periosteal initial mineralization rate (% of max/hr). Previous studies demonstrated that after injection tetracycline diffuses into bone which has attained about 20% of its maximum mineral concentration, but no further. The time required to reach 20% of maximum mineralization is determined by converting the width of this initial band of tetracycline uptake to time, using the relationship given by the bone apposition rate. The mineral concentration divided by this time yields this rate.

G) Medullary cavity area (mm^2). This parameter is used to estimate the endosteal bone resorption since endosteal bone formation could not be measured. A small amount of resorption occurs around vascular canals but this is only about 10% of the total (8) and is ignored. Thus, medullary area underestimates bone resorption rate; the amount of error is directly related to the amount of endosteal bone formed.

4) Bone length measurements were made with calipers.

PRECISION AND STATISTICAL ANALYSIS

The precision of the quantitative histologic measurements made in this study is $\pm 3\%$ or less (9). The precision of measurements of pore size distribution is 2-3% (6). Correlation, regression, and covariance analysis were made by means of a PDP 8 computer and DECUS programs which are based on standard statistical methods (11).

RESULTS

Space flight had little effect on the bone porosimetry parameters measured, although the synchronous controls were quite different from the vivarium animals (Table 1). The flight animals did show a significant decrease in bone density as compared with the vivarium but not the synchronous controls immediately following flight (R+0). An increase in the lacunar-canalicular volume, which is indicative of osteocytic activity, did occur in both the synchronous and flight animals immediately after flight; however, the latter group did not show a significant decrease from the vivarium controls due to a high variance. During the recovery period (R+25), the only significant value noted was an increase in lacunar-canalicular pore diameter in the flight animals as compared with the vivarium controls.

Breaking strength measurements show differences in mean values (Table 2). However, due to large scatter, significant differences were not observed in any of the parameters.

The most striking change in the flight animals at R+0 was the decreased rate of periosteal bone formation which was approximately 40% less than the vivarium and synchronous controls (Table 3, Fig. 3). In fact, all bone parameters associated with formation and mineralization were decreased significantly in the flight animals at R+0; these decreases probably contributed to the slight increase in medullary area. A significant finding was the occurrence of an arrest line at both the periosteum and endosteum of the flight animals (Fig. 4a). Although the synchronous controls showed some indication of an arrest line at the periosteum, it was not as extensive as the flight group and did not occur at the endosteum (Fig. 4b). The vivarium controls showed no signs

TABLE 1

EFFECT OF SPACEFLIGHT ON BONE POROSIMETRY

	BONE DENSITY g/cc	LACUN.-CANALICUL. PORE DIAMETER, μ (at peak vol.)	VOLUME, cc/g $\times 10^{-3}$			TOTAL POROSITY cc/g $\times 10^{-3}$
			LACUN.-CANALICUL.	LARGE CANALICUL.	VASCULAR CANAL	
R+0						
FLIGHT	1.475 $\pm$.032**	0.199 $\pm$.002	20.46 $\pm$ 1.71	4.73 $\pm$.64	37.5 $\pm$ 4.9	67.0 $\pm$ 5.9
SYNCHRONOUS	1.490 $\pm$.022*	0.203 $\pm$.002**	20.39 $\pm$.59**	5.81 $\pm$.76**	47.4 $\pm$ 11.6	78.5 $\pm$ 12.7**
VIVARIUM	1.513 $\pm$.029	0.198 $\pm$.002	19.02 $\pm$.42	4.74 $\pm$.17	38.7 $\pm$ 3.1	67.0 $\pm$ 2.9
R+25						
FLIGHT	1.562 $\pm$.017	0.195 $\pm$.005**	18.58 $\pm$ 1.20	4.97 $\pm$.56	34.3 $\pm$ 6.5	61.7 $\pm$ 6.1
SYNCHRONOUS	1.562 $\pm$.047	0.191 $\pm$.007	18.32 $\pm$ 1.03	4.75 $\pm$.76	37.4 $\pm$ 4.1	64.7 $\pm$ 4.1
VIVARIUM	1.554 $\pm$.022	0.191 $\pm$.002	18.18 $\pm$.86	4.71 $\pm$.49	39.3 $\pm$ 7.7	66.1 $\pm$ 8.9

* mean $\pm$ S.D. for 6 rats

** significantly different from vivarium controls

TABLE 2

EFFECT OF SPACEFLIGHT ON HUMERUS BREAKING STRENGTH

	n	TORQUE (dyneX10 ⁵ -cm)	DEFORMATION (degrees)	ENERGY (dyneX10 ⁵ -cm radian)	STIFFNESS (dyneX10 ⁵ -cm/radian)
R+0					
FLIGHT	4	6.3 $\pm$ 3.2*	12.0 $\pm$ 5.7	0.82 $\pm$ 0.65	1.31 $\pm$ 0.31
SYNCHRONOUS	5	6.6 $\pm$ 4.1	17.0 $\pm$ 6.7	1.18 $\pm$ 1.12	0.95 $\pm$ 0.29
VIVARIUM	4	5.8 $\pm$ 2.0	11.8 $\pm$ 2.7	0.59 $\pm$ 0.25	1.13 $\pm$ 0.35
R+25					
FLIGHT	4	5.6 $\pm$ 2.9	11.3 $\pm$ 3.0	0.64 $\pm$ 0.45	1.08 $\pm$ 0.31
SYNCHRONOUS	6	9.1 $\pm$ 7.0	13.5 $\pm$ 6.4	1.42 $\pm$ 1.55	1.34 $\pm$ 0.43
VIVARIUM	6	14.0 $\pm$ 8.6	23.2 $\pm$ 12.7	3.60 $\pm$ 3.15	1.27 $\pm$ 0.41

n = number of rats/group

* mean $\pm$ S.D.

TABLE 3

EFFECT OF SPACEFLIGHT ON PARAMETERS OF BONE TURNOVER

GROUP	<u>FLIGHT</u>			<u>RECOVERY</u>	
	FLIGHT	VIVARIUM	SYNCHRONOUS	FLIGHT	VIVARIUM
N	11	4	7	5	4
PERIOSTEAL FORMATION ($\text{mm}^3/\text{day} \times 10^3$)					
BONE	9.4 $\pm$ 2.8*	16.0 $\pm$ 1.4**	15.8 $\pm$ 1.5	17.1 $\pm$ 2.2*	11.3 $\pm$ 1.4
MATRIX	7.2 $\pm$ 2.8*	14.0 $\pm$ 1.4	13.8 $\pm$ 1.4	17.8 $\pm$ 2.1*	11.2 $\pm$ 1.4
PERIOSTEAL APPPOSITION ($\mu/\text{day} \times 10^3$)					
BONE	1.3 $\pm$ 0.4*	2.1 $\pm$ 0.2	2.2 $\pm$ 0.2	2.2 $\pm$ 0.2*	1.4 $\pm$ 0.2
MATRIX	1.0 $\pm$ 0.4*	1.8 $\pm$ 0.2	1.9 $\pm$ 0.2	2.3 $\pm$ 0.2*	1.4 $\pm$ 0.2
PERIOSTEAL OSTEOID MATURATION RATE (%/hr)	0.58 $\pm$ 0.23*	1.01 $\pm$ 0.09	1.11 $\pm$ 0.11	2.30 $\pm$ 0.34*	1.41 $\pm$ 0.28
PERIOSTEAL INITIAL MINERALIZATION RATE (% max/hr)	0.41 $\pm$ 0.13*	0.68 $\pm$ 0.05	0.64 $\pm$ 0.06	0.81 $\pm$ 0.13*	0.56 $\pm$ 0.09
N	6	6	6	6	6
MEDULLARY AREA (mm^2)	0.96 $\pm$ 0.22	0.86 $\pm$ 0.11	0.86 $\pm$ 0.10	0.82 $\pm$ 0.07	0.90 $\pm$ 0.12

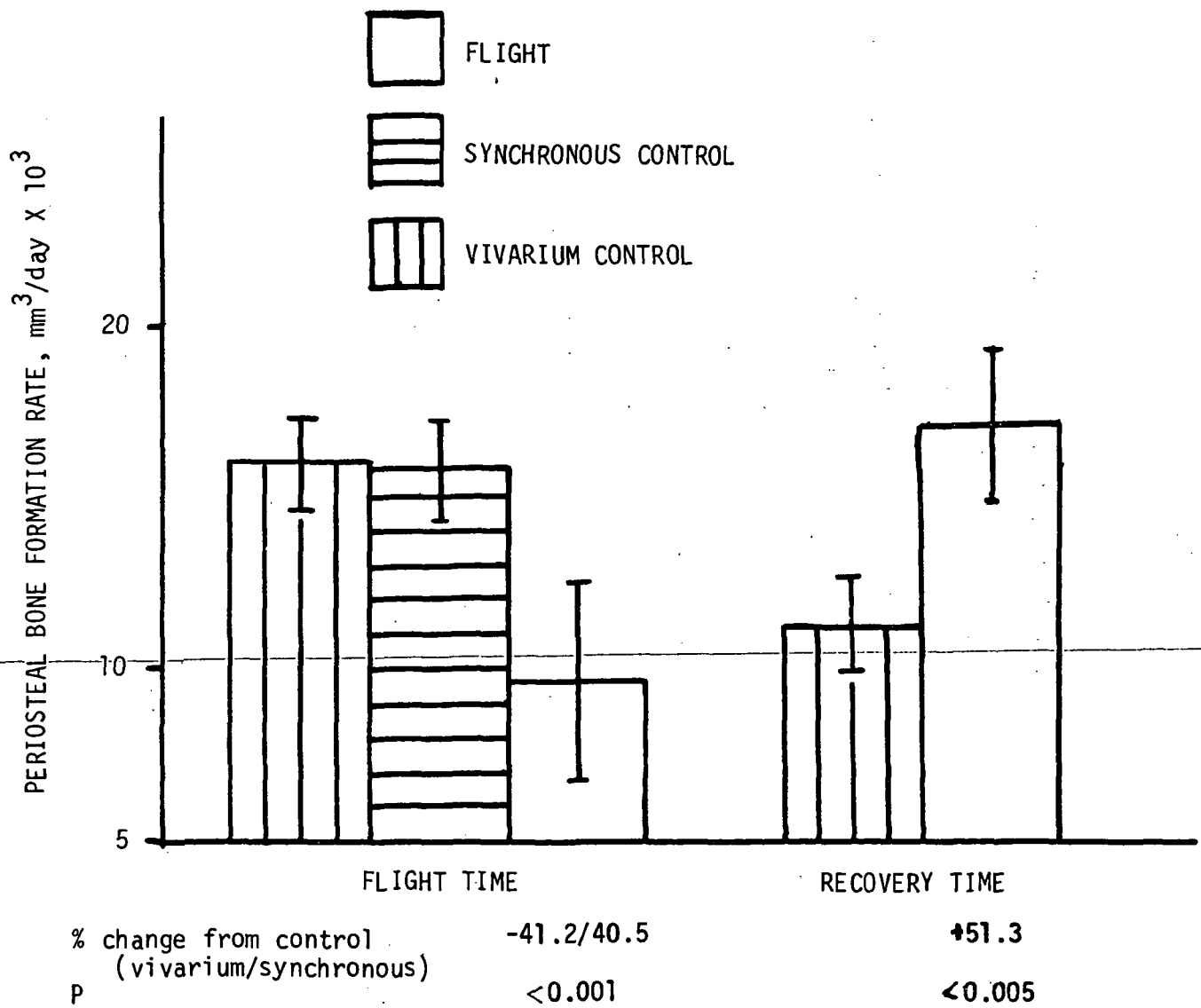
N=number of rats/group

*=significantly different from control groups

**=mean $\pm$ S.D.

FIGURE 3

EFFECT OF SPACEFLIGHT ON PERIOSTEAL BONE FORMATION RATE



of an arrest line at either surface (Fig. 4c). When an enlarged Brightfield photomicrograph from the flight group at R+25 (Fig. 5a) is compared with the same photomicrograph under UV light (Fig. 5b), one can see that the tetracycline label is superimposed on the arrest line. A reversal line, which is much more irregular, can be seen at the endosteum; this line, however, is not associated with the tetracycline label. When these sections were stained for acid phosphatase, the reversal line exhibited enzyme activity while the arrest line did not.

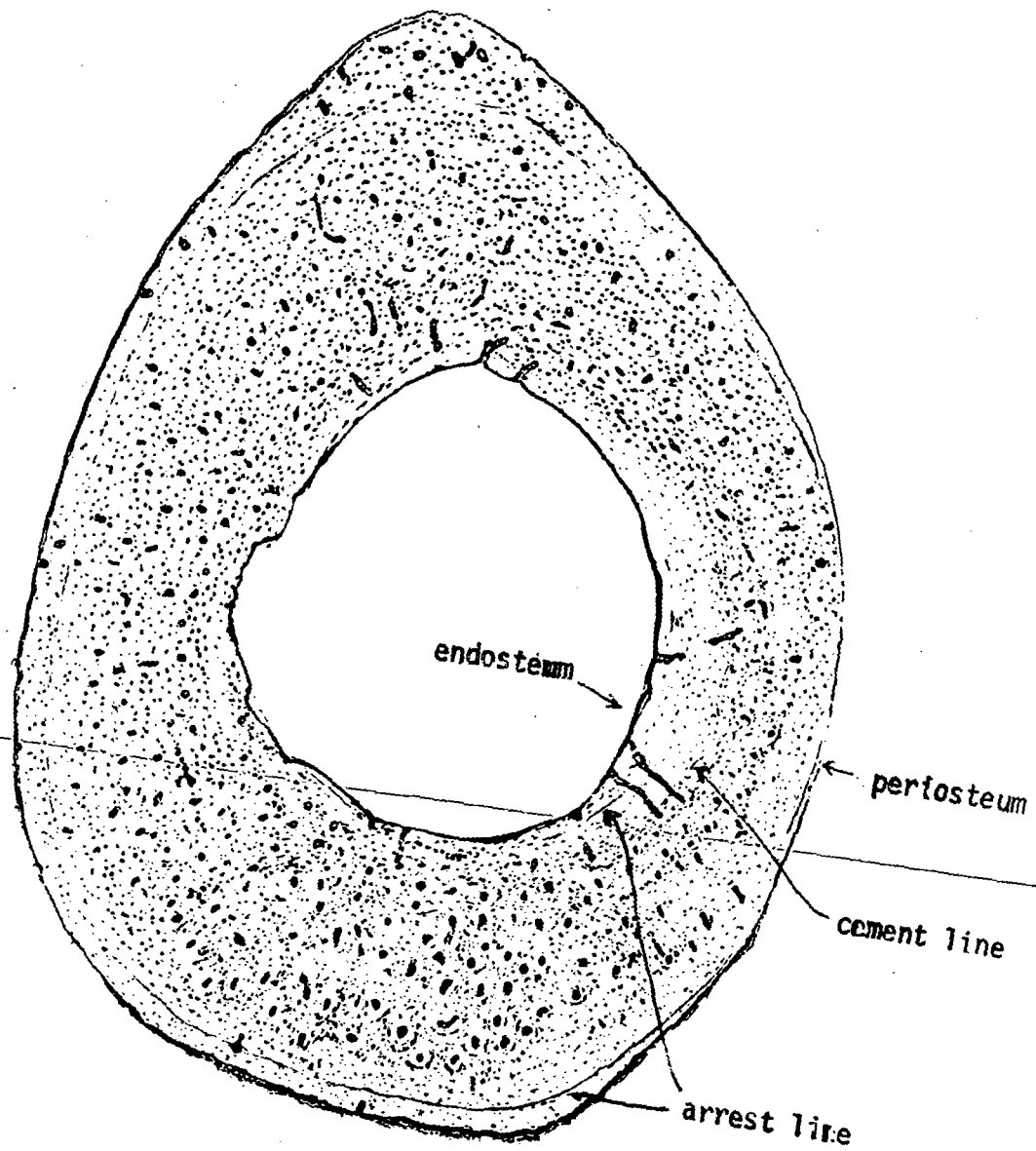
The larger number of rats at the R+0 period (Table 3) is due to the addition of data from the first labeling period of the R+25 animals. Of importance is the finding that in all the flight animals the linear bone formation rate was less in the R+0 period than at R+25 and that none of the data from the R+0 rats overlapped with that at R+25.

During the recovery period, the flight animals significantly increased all parameters associated with formation when compared with either the vivarium controls or the R+0 flight data (Table 3, Fig. 3). The magnitude of this increase was about 50%, similar to the decrease noted in the R+0 rats (Fig. 3). Only 2 synchronous controls were injected with tetracycline for analysis and the data obtained were not reliable.

Osteoblastic and osteoclastic cell population measurements were attempted at the endosteum and periosteum. Unfortunately, obvious artifacts at the endosteum (probably due to marrow clot retraction) and stripping of the periosteum during preparation of the specimens made definitive cell counts impossible. Indications of osteoblasts along the arrest line and unchanged osteoclast populations at the endosteum were noticed in the better sections, but the data is only suggestive.

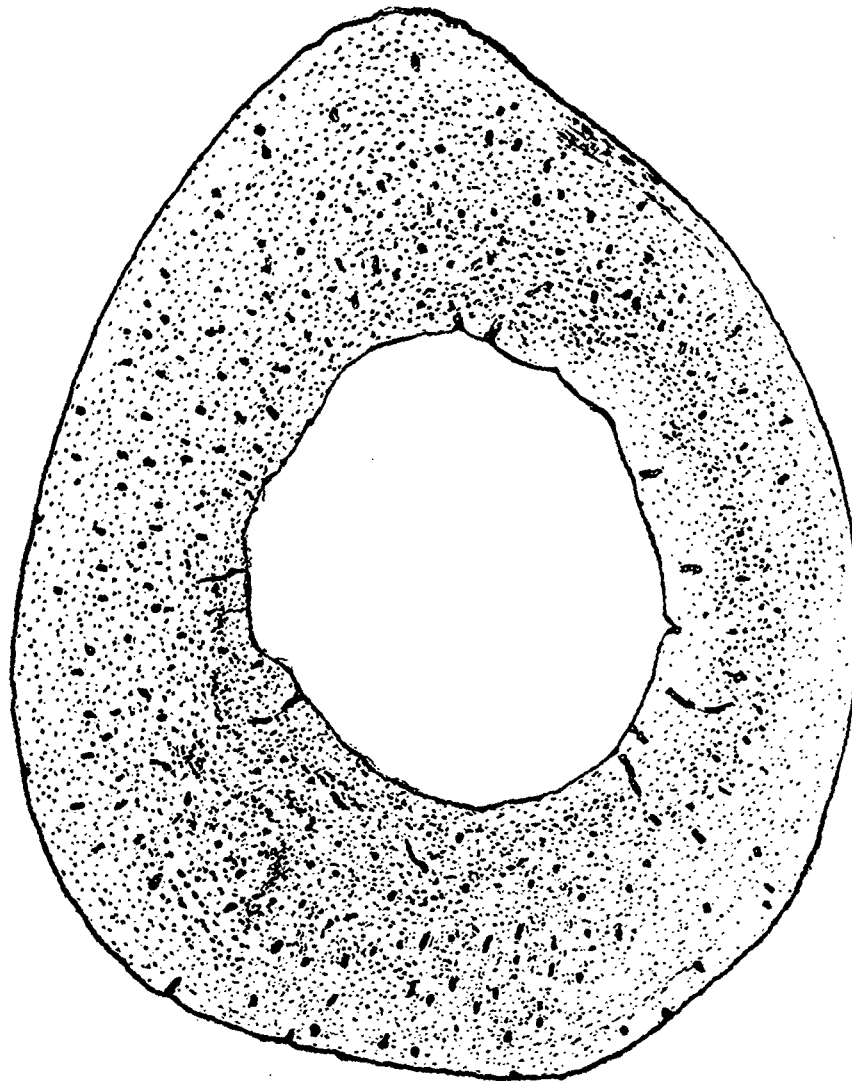
The body weights of the rats and the length of the left humerus and tibia

FIGURE 4a.
PHOTOMICROGRAPH OF A TIBIAL GROUND SECTION
OF A FLIGHT RAT



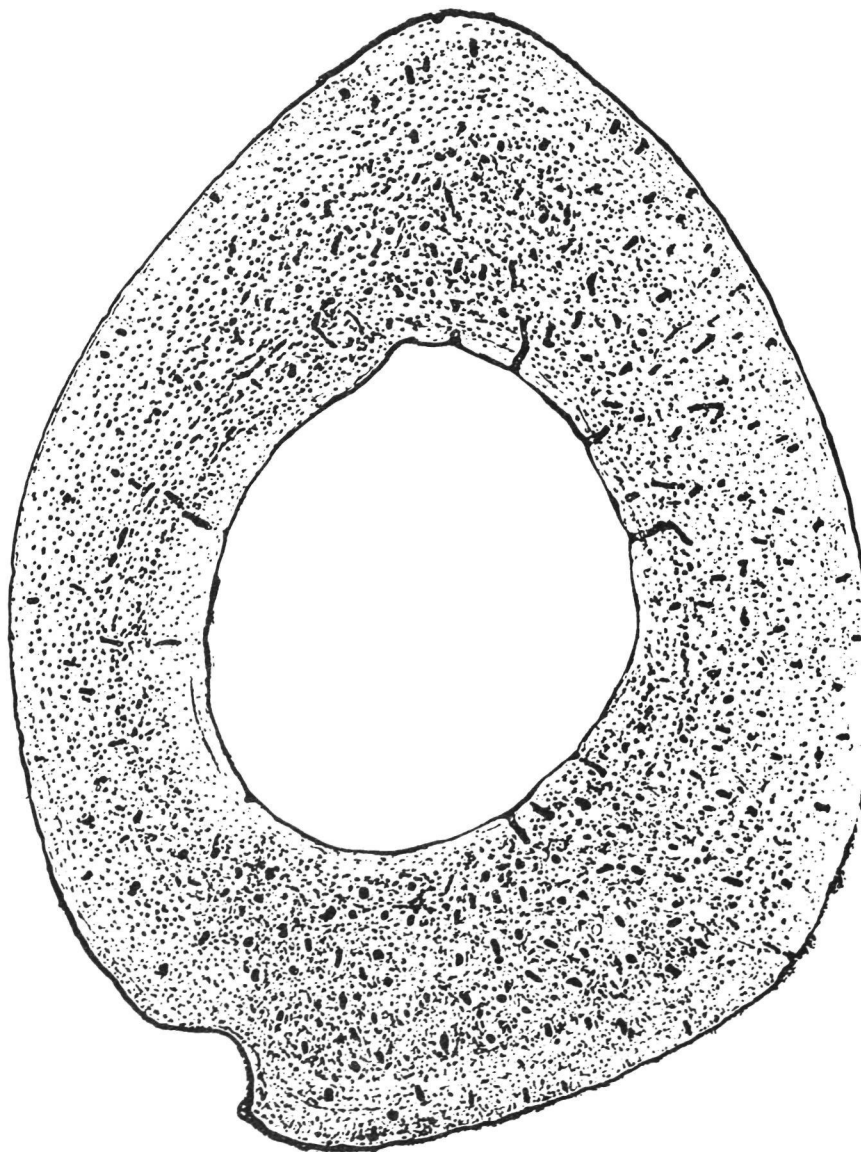
The photomicrograph was from a flight rat sacrificed at R+25. An arrest line can be seen around the periosteum and partially around the endosteum.

FIGURE 4b
PHOTOMICROGRAPH OF A TIBIAL GROUND SECTION
OF A SYNCHRONOUS CONTROL RAT



The photomicrograph was from a synchronous control rat sacrificed at R+25.
A suggestion of an arrest line is seen along the lower right periosteum.

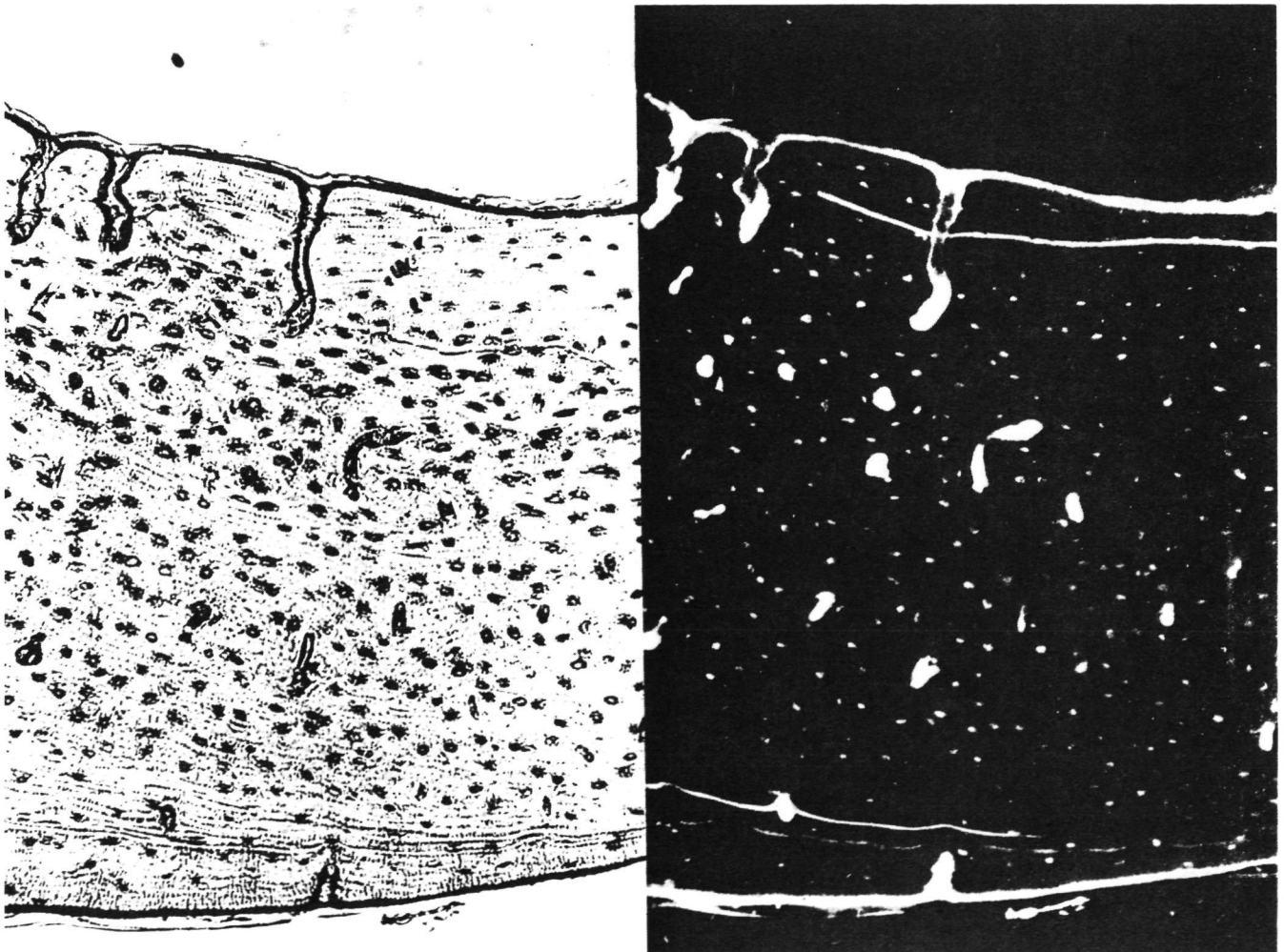
FIGURE 4c
PHOTOMICROGRAPH OF A TIBIAL GROUND SECTION
OF A VIVARIUM CONTROL RAT



The photomicrograph was from a vivarium control animal sacrificed at R+25. No suggestion of an arrest line is seen at either the periosteum or endosteum.

FIGURE 5

ENLARGED PHOTOMICROGRAPH OF A SECTION OF TIBIAL
DIAPHYSIS FROM A R+25 FLIGHT RAT



A. Brightfield

B. Ultraviolet

are found in Table 4. No statistically significant difference was noted in either body weight or bone length at R+0 or at R+25. Humerus length is very well correlated with body weight ($r=0.92$) while no such correlation was noted with the left tibia (Fig. 6). No differences were found in tibia cross sectional area at R+0, but the flight and synchronous rats at R+25 had significantly smaller cross sectional areas than did the vivarium controls.

TABLE 4
BODY WEIGHT AND BONE DIMENSIONS

	n	WEIGHT (gm)	LEFT HUMERUS LENGTH (cm)	n	WEIGHT (gm)	LEFT TIBIA LENGTH (cm)	TIBIA CROSS SECTION AREA (mm ²)
R+0							
FLIGHT	4	258 $\pm$ 4*	2.56 $\pm$ 0.02	5	262 $\pm$ 9	3.82 $\pm$ 1.36	4.18 $\pm$ 0.26
SYNCHRONOUS	4	269 $\pm$ 13	2.53 $\pm$ 0.04	5	267 $\pm$ 13	3.78 $\pm$ 0.51	3.92 $\pm$ 0.31
VIVARIUM	4	274 $\pm$ 16	2.63 $\pm$ 0.05	5	269 $\pm$ 13	3.90 $\pm$ 0.86	4.07 $\pm$ 0.22
R+25							
FLIGHT	4	332 $\pm$ 15	2.73 $\pm$ 0.05	6	328 $\pm$ 14	3.83 $\pm$ 0.55	4.31 $\pm$ 0.26**
SYNCHRONOUS	6	325 $\pm$ 10	2.75 $\pm$ 0.04	6	325 $\pm$ 10	3.82 $\pm$ 0.90	4.32 $\pm$ 0.23**
VIVARIUM	6	331 $\pm$ 15	2.77 $\pm$ 0.03	6	331 $\pm$ 15	3.86 $\pm$ 0.53	4.70 $\pm$ 0.19

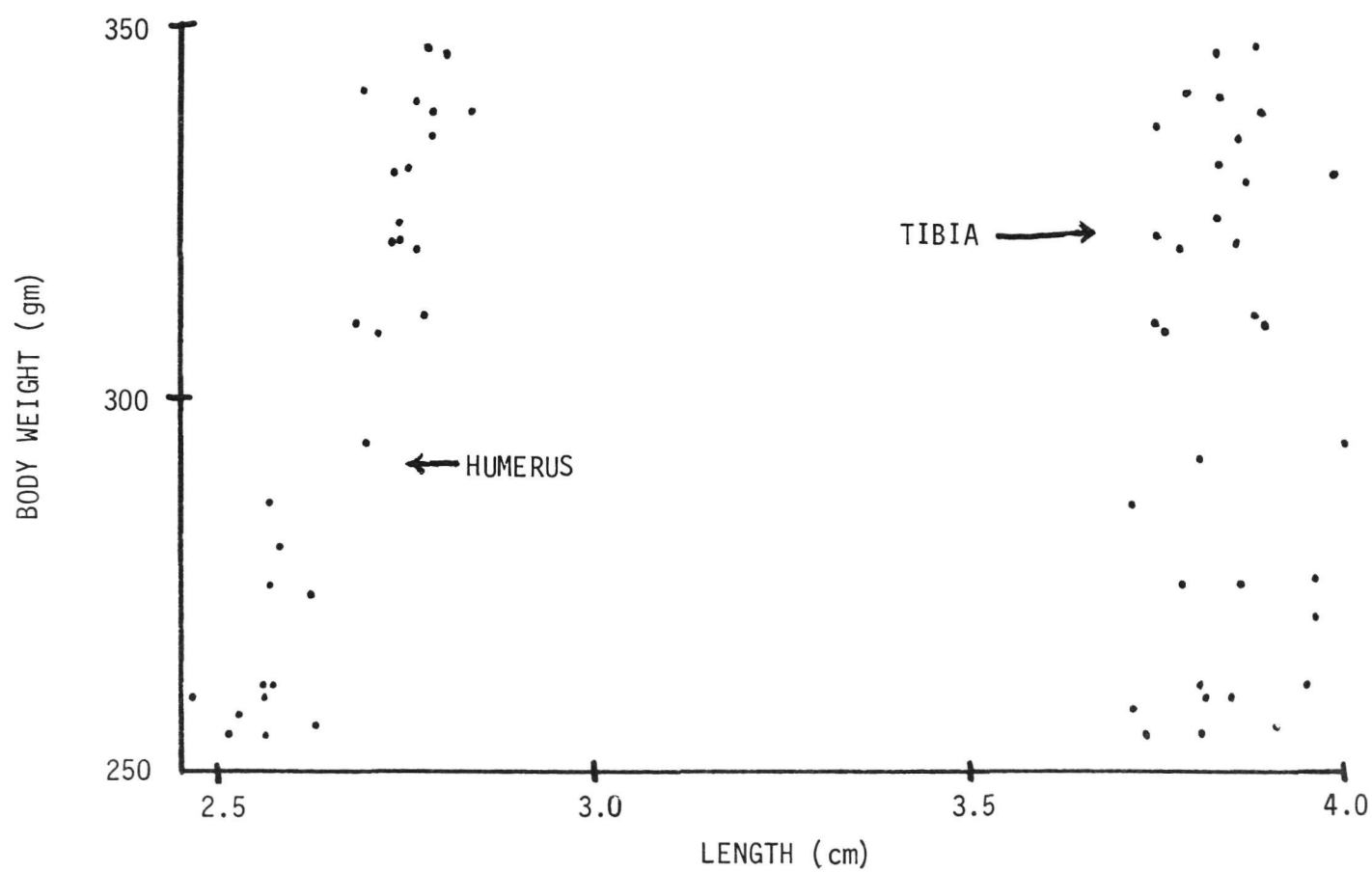
n = number of rats/group

* mean $\pm$ S.D.

** significantly different from vivarium controls

FIGURE 6

BODY WEIGHT VS BONE LENGTH*



*Pooled data from all groups at both time periods, R+0 and R+25

DISCUSSION

Space flight has a pronounced effect on bone in young, growing rats. The most striking effects are highly significant decreases in the rates of periosteal formation, apposition, osteoid maturation, and mineralization (Table 3, Fig. 3). The changes are reflected in some of the other bone parameters measured.

The decrease in bone density noted in the flight animals (Table 1) may reflect the decrease in the rate of mineralization (Table 3). A change in osteocytic activity is indicated by the increase in lacunar-canalicular volume (Table 1) and would be consistent with a decrease in formation or an increase in bone resorption. After a 25 day recovery period, an increase in density and cross sectional area and a decrease in porosity, lacunar-canalicular volume, and medullary cavity area in the flight animals as compared with the flight data suggests that bone mechanisms were able to rapidly correct or even overcompensate for the effects of space flight (Tables 1, 2, and 3). The significant increase in bone porosity and pore size distribution in the synchronous animals suggests that space flight simulation had a greater effect on these parameters than did space flight.

The effect of space flight on bone mechanical properties is difficult to assess due to the high variations (Table 2). One might have expected a decrease in mechanical strength from the porosimetry and formation data (Table 1). Since several of the bones were fractured upon arrival, some of the problems associated with these analyses might have been due to microfractures created during handling; microfractures act as stress concentrators and cause great variance. Also, the humerus due to its more triangular shape does not have the geometrical features of a hollow

cylinder which are necessary for testing whole bone mechanical properties and, therefore, it is not an ideal bone for studying breaking strength. Slight variations in the position of the supracondylar ridge during testing might alter mechanical properties. Although the femur was originally requested for this study, concern about the effect of a 3% acetic acid wash on bone demineralization necessitated a shift in specimens. The femur is a better bone for assessing changes in mechanical properties caused by space flight since its geometry conforms to a hollow cylinder and since it is a weight-bearing bone. In fact, Stupakove et al. (personal communications) found a 30% decrease in bone strength in the femur of rats flown on Cosmos 782.

The dramatic decrease in formation (Table 3, Fig. 3) was accompanied by an arrest line at both the periosteal and endosteal surfaces (Fig. 4a and 5). The arrest line is a smooth, distinct demarcation unlike the reversal or cement line which is often found at the endosteum (Fig. 5a) and is very irregular; the former is created when formation ceases and is later reinitiated while the latter occurs when resorption reverses to formation. The arrest of bone formation is probably due to a cessation of either osteoblast activity or osteoblast cell proliferation. Indications of osteoblasts were found along the periosteum in the few histological sections which had no noticeable artifacts suggesting that the cells were quiescent; these data need to be confirmed. A decrease in linear formation is obvious in the UV photomicrograph (Fig. 5b); the distance between the tetracycline labels (R+0) is considerably less than the distance from the second label to the periosteum (R+25). Also, the second tetracycline label is much fainter than the first suggesting that mineralization had only recently been reinitiated. If the osteoblasts

along the arrest line were indeed quiescent, a stimulus such as reentry may have signaled the osteoblasts to once again form osteoid; this osteoid would then have to mature before mineralization could occur. If the animals had been injected with tetracycline immediately postflight, the drug may not have labeled since mineralized bone is necessary for tetracycline deposition and retention.

A decrease in bone mass during flight was not totally unexpected since similar results have been found in immobilized animals (12, 13, 14) and man (4, 5) and in rats (1) and man (2) following space flight. This decrease could be related to non-specific factors (such as decreased growth or food intake), to endocrine alterations, or to decreased mechanical stress. Non-specific factors can be ruled out since body weight and tibia length and cross sectional area did not differ from the control animals (Table 4). Hormones primarily involved with calcium homeostasis, parathyroid hormone (PTH), vitamin D metabolites, and calcitonin (CT), do not seem to be major factors since no changes in parathyroid or thyroid gland histology (Plakhuta-Plakutina, personal communications) or serum calcium (Tigranyin, personal communications) were noted in the flight rats. Also, no change in PTH, CT, or 25-hydroxyvitamin D₃ was found in the Skylab astronauts (15). However, the Skylab astronauts excreted significantly higher levels of cortisol, 17-ketosteroids, and calcium. Similarly, the flight rats had significantly larger adrenal glands due primarily to increased corticosterone content (Portugalov et al., personal communications). The glucocorticoids have been shown to depress protein synthesis (16, 17) and to cause the loss of bone volume (18). They also consistently decrease bone formation and the number of osteoblasts regardless of dose (19). Cortisol appears to favor the morphogenesis of precursor cells to osteoclasts in rats, but the effect

is dose-dependent with low doses stimulating and high doses suppressing cell division and osteoclast number. The low dose effect may be mediated through the parathyroids (19, 20). Thus, the overall effect of the glucocorticoids is to decrease bone formation and the number of osteoblasts and osteoclasts, changes similar to the results suggested by this study. The suppression of bone formation was seen in the tibia which is a weight-bearing bone. Since the decrease in bone density in the Skylab astronauts was found only in a weight-bearing bone, the os calcis, and not in the distal radius and ulna (2), changes in bone loading must also be important. Many works have stressed the importance of mechanical forces on skeletal tissues (3, 21, 22). Years ago, Albright (23) hypothesized that the osteoblast was dependent upon mechanical forces for stimulation of bone formation and maintenance of bone mass. Mechanical forces or glucocorticoids or both may influence bone turnover during flight. Whatever the mechanism(s), bone formation is definitely suppressed during flight; the effect of space flight on resorption, although probably not as great, has not yet been quantified.

The key finding of this study is that the R+25 flight animals consistently and substantially formed less bone at the periosteum during flight than during recovery. Less certain is whether the amount of bone formed during recovery in the flight animals was increased over the vivarium group because of the confusion in the labeling dates. The first finding probably requires no qualifications, but the latter (the rebound) is less certain. To further define the effects of space flight on bone and to clarify some of the findings in this study, the experiment will be repeated in 1977.

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COSMIC RAY EFFECTS ON THE EYES OF

RATS FLOWN ON COSMOS 782

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Abstract

The eyes from six rats were fixed at the recovery site in Russia after circling the earth for 19.5 days in a 62.8° orbit. Twelve more flight eyes were fixed 25 days later. These two preparations and eyes exposed to 1000 rads of neon and argon, were compared to obtain data on possible radiation effects on the retina. The outer nuclear layer was examined for radiation changes because these nuclei control the synthesis of the outer segments. Here, any interference with the synthesis would be reflected by an alteration in morphology. Most of the flight eye tissue was normal indicating spaceflight for this duration of postflight period would be safe for passengers and crew, but these studies could not be conducted beyond 25 days. Necrotic nuclei were found in the outer nuclear layer and channels were located in the outer segment area. Macrophages were seen between the pigment layer and outer segments. Comparison of the zero-day and 25-day postflight eyes suggested some possible recovery. Light flashes seen by space travelers and damage from cosmic rays appear to arise from two different sites of interaction. The flashes are created by cosmic ray traversal of the outer segments while pathology, when it occurs, is quite possibly from interaction with some part of the nucleus. Nevertheless, direct interaction on cellular components could also occur. It appears that a low dose effect may have been seen on the flight, lower than one would assume from Berkeley experiments. Other factors such as secondaries from spacecraft shielding may play an important role.

Introduction

A prediction was made in 1952 by Tobias (18) that "light flashes" would be seen by dark-adapted persons in high flying aircraft and during space flight. Tobias correctly deduced that cosmic rays (stripped nuclei of the various atoms) would pass through and actuate the photoreceptors of the retina. The Apollo 11 mission (17) confirmed this prediction and stimulated interest in determining the possible biological effect of these particles. Subsequently, the same flashes were seen by persons who exposed their retinas to cyclotron beams (2, 3, 7, 13, 20). The brain and optic nerve, however, did not respond when irradiated to produce these flashes (3, 19). Direct ionization in the retina has been thought to be the cause of the flashes (19). Cerenkov radiation has also been implicated as a contributor, possibly being produced as the high atomic number, high energy (HZE) particles traverse the vitreous (8, 9, 10). The production of some HZE particles at the Lawrence Berkeley Laboratory, and the flight of Apollo 17 (15) provided a chance to study their effect on the eye (1, 11, 12, 14, 16, 21). Further evidence for HZE particle activation of the photoreceptors was obtained with the first ERG using rabbit retina and nitrogen nuclei (14, 16). Ultrastructural alterations were also observed in the outer segments and cells of the pigment epithelium in pocket mice retinas (16). Blood vessel alterations were demonstrated in monkeys using Fundus photography (1). Recent neon and argon exposures have also revealed (+90 day report) alterations in the outer nuclear layer, inner and outer rod segments, pigment epithelium, and blood vessels. The

site of sharpest vision is in the rod free area of the fovea, the tiny central area of the macula. Cellular damage in this area of best vision (which is about 260 μm in diameter) could become a severe problem by limiting vision in space travelers. In addition, the possibility of cataract formation must also be considered because long-term, low-dose exposure could cause lens changes. Although data are lacking in this area of long-term, low-dose response, the possibility of extended flights makes such information vital.

Materials and Methods

Twenty-six white rats, Wistar strain, were flown by the Russians on their Cosmos No. 782 satellite. The flight lasted 19.5 days and circled the earth in a 62.8° orbit from November 25 to December 15, 1975. Radiation dosimeters were placed aboard the spacecraft (Benton, G., R + 90 day report on dosimetry onboard Cosmos No. 782) to obtain an average flux and atomic number of the cosmic ray particles. The rats did not have implanted monitors during the flight. Two types of controls, synchronous and vivarium, were maintained in Moscow during the period of flight. The temperature and gas mixture data within the satellite were transmitted to the ground and 5 days later the synchronous group of animals were subjected to the same conditions. These rats were also subjected to simulated blast-off and recovery stress. The vivarium rats were maintained under usual laboratory conditions to provide completely unstressed controls. Animals were always sacrificed at the same time of day to obviate any circadian rhythm effect.

Each eye from six flight rats was enucleated at the recovery site, and almost immediately after decapitation was placed in a dish of cold "triple fix" (6) and oriented with the lens down and the optic nerve up. An eyeball-shaped depression formed in silastic provided mechanical support while a sharp razor blade was lightly drawn across the back of the eye. This opened the eye, allowing immediate entry of the triple fix. Then a fine eye scissors was used to further open the eye to insure complete fixation. A pipet was gently inserted through the slit near the optic nerve and a very slow flow of fixative was introduced through it, directing the flow against the lens to further reduce the velocity. Each eye was then placed in a small specimen bottle; 30 min after the initial processing the bottles were gently agitated to insure that fresh fixative reached the retinas. The entire operation took less than 2 min. The eyes were stored at 4° C in the triple fix. This fixation procedure was repeated on another six flight rats 25 days after the flight. Eyes were also taken from six synchronous and six vivarium control rats, both immediately after the flight and 25 days later, and prepared in the same way. In order to transport the tissue back to the United States, the eyes were transferred to bio-transporters, packed in ice, and flown from Moscow to Ames Research Center. To insure that the specimens were maintained upright and refrigerated for the entire trip, a courier accompanied the bio-transporters.

Prior to the Cosmos flight, a set of six rat eyes was opened and fixed in the same manner, then stored at 4° C for 1 week. They were flown to New York and returned for processing.

All of the eyes were post fixed in 1% osmium and very slowly dehydrated and embedded in plastic. Each eye provided two embedded blocks because the preparation procedure had already partially separated the eye into two halves. Sections from each hemisphere were cut at 2 μ m for light microscopy and examined by phase contrast microscopy without being stained; other sections were examined after silver staining (4, 5) or silver-gold staining (C. Turnbull, unpublished). Sections were cut for electron microscopy for either routine examination or whenever light microscopy indicated areas of interest. The sections for electron microscopy were either mounted on large mesh grids or on single-hole grids coated to add support for the large sections. Photographs of large retinal areas of both flight and control tissues were glued together and the resulting composites compared. These tissues were also compared with retinas that had been neon- or argon-exposed in the Lawrence Berkeley Bevelac and with the eyes flown on the round trip to New York.

Results

The ultrastructure of the rat eyes, which were prepared in the same manner as the Russian rat eyes and flown to New York, appeared normal and no alterations from the commercial handling or the jet flight could be detected. Transportation of the eyes immersed in triple fix, and placed in small bottles with the lens still in place, restricted movement within the eye and also prevented its collapse. However, even though splitting the sclera near the optic nerve provided rapid fixation, movement of the razor blade across the soft adjacent retina resulted in detachment near the slit which disrupted

the outer segments. Consequently, the slit was made with the slightest possible pressure and further opening was done with a fine pair of eye scissors. This reduced the detached area. A further reduction in detachment was achieved with very slow and gentle dehydration.

The flux of HZE particles, measured by Gene Benton's dosimeters, indicated an average of 80 particles/cm² for the 19.5 day flight with Z numbers between 6 and 28. Using this statistical average of 80 particles/cm² applied to the cross-sectional area of the rat eye, an average of ≈ 30.8 particles could have traversed a flat retina (using a diameter of 7 mm, the retinal surface area would be 1.54 cm² and multiplying by 80 p/cm² = 123 particles entering the eye).

The retinas of pocket mice and C-57 black mice exposed to neon and argon particles in the Bevelac at Lawrence Berkeley will be reported in detail in another publication. However, the 1000 rad exposures resulted in positive alterations, thus providing comparison data for the Cosmos flight eyes. The cells of the outer nuclear layer appeared to be the most affected by the high Z-particle radiation (Figs. 1, 2). Occasionally, cells in the outer nuclear layer became swollen with clearing of the cytoplasm. In some instances, the inner segments directly above these necrotic cells were also swollen and contained myelin figures and swollen mitochondria; this suggests that they might be extensions from the same cell (Fig. 2). Disruption of the outer segments also occurred. Channels of clear areas, approximately the diameter of a cell, were followed by serial sectioning and light microscopy. These channels appeared in the neon and the argon exposures and varied in length from 10 to about 15 μm . The

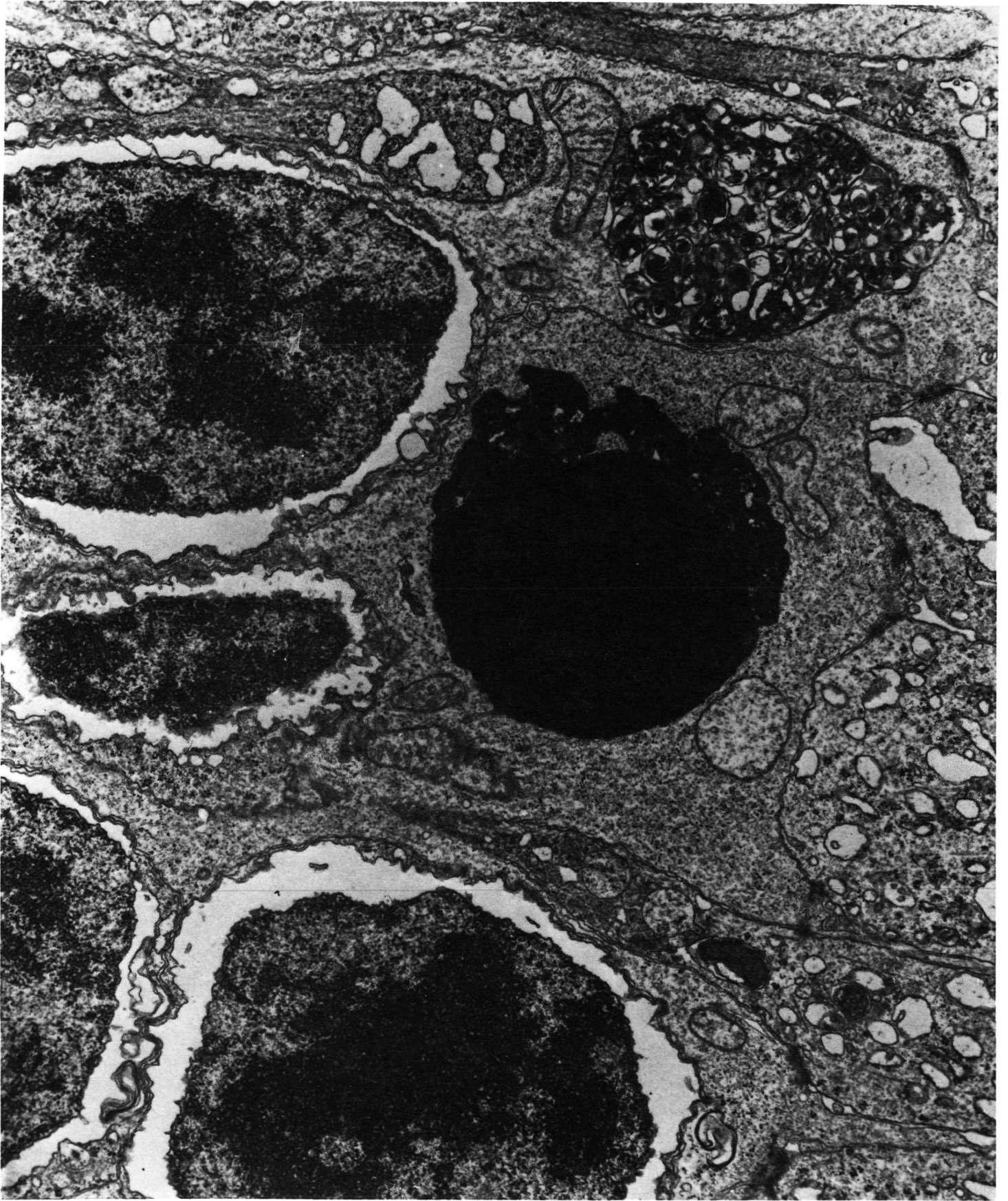


Fig. 1. Mouse retina exposed to 1000 rad argon. Note the dark necrotic nuclei and adjacent abnormal cellular debris. 19,500 X.

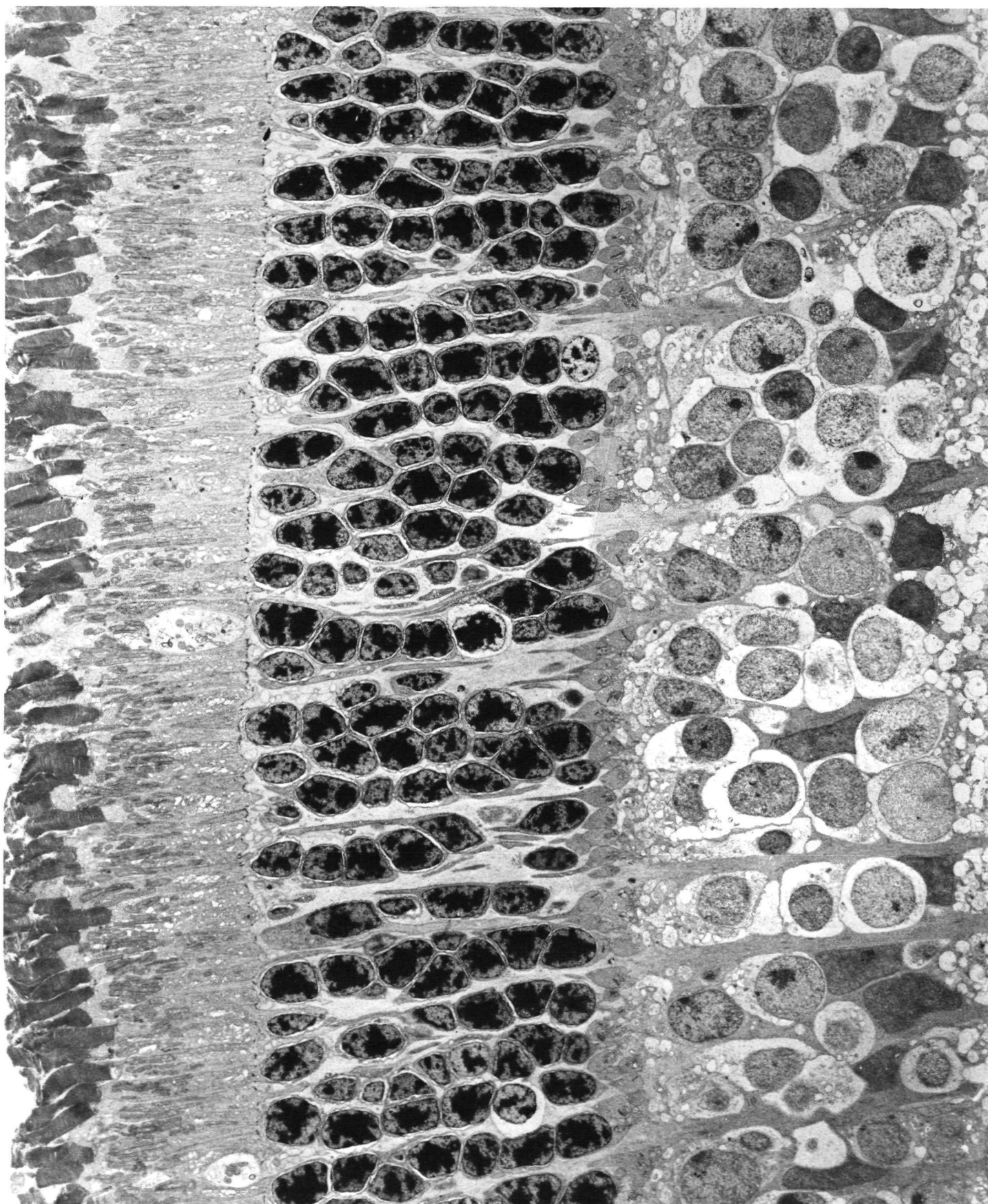


Fig. 2. Mouse retina exposed to 1000 rad of argon. Note the two
necrotic nuclei below two necrotic inner segments. 1900 X.
360

channels appeared to be straight. Fewer but occasional cells in the inner nuclear layer were also swollen with clear cytoplasm. Such cells were visible in both the light and electron microscope. One rod area subjected to the argon beam had free floating particles adjacent to the saccules of the rods. This, however, was not a general finding.

The 2- μ m sections of the plastic embedded eyes provided excellent material for phase contrast microscopy and silver staining (4, 5) and resulted in increased sharpness of detail.

A few nuclei from the outer nuclear layer were displaced into the inner segment area apparently by passing through the outer limiting membrane. An increase of displaced nuclei was seen after argon irradiation relative to neon exposures. An increase in platelets also occurred in the retinal blood vessels.

Examination of the flight eyes revealed mostly normal tissue, although a few alterations were seen. Occasional necrotic nuclei occurred in the outer and inner nuclear layer (Figs. 3-5). One outer nuclear cell body was displaced into the inner segment area (Fig. 6). Channels, comparable to, but larger than those seen after argon and neon radiation, were also observed (Figs. 7, 8), especially at the junction of the rod-pigment epithelium layer. Two macrophages were discovered in a channel at the junction (Figs. 9, 10). This channel ran for 26 μ m; another channel, which was adjacent, ran for 14 μ m (Fig. 8). At least four more channels were present in this eye. Some of the inner segments contained cytoplasmic debris, large vesicles (Figs. 11, 12), and altered mitochondria (Fig. 13). The rods were often disrupted, the disruption probably caused by the handling procedures necessary for preparation. However, a few rods were abnormal

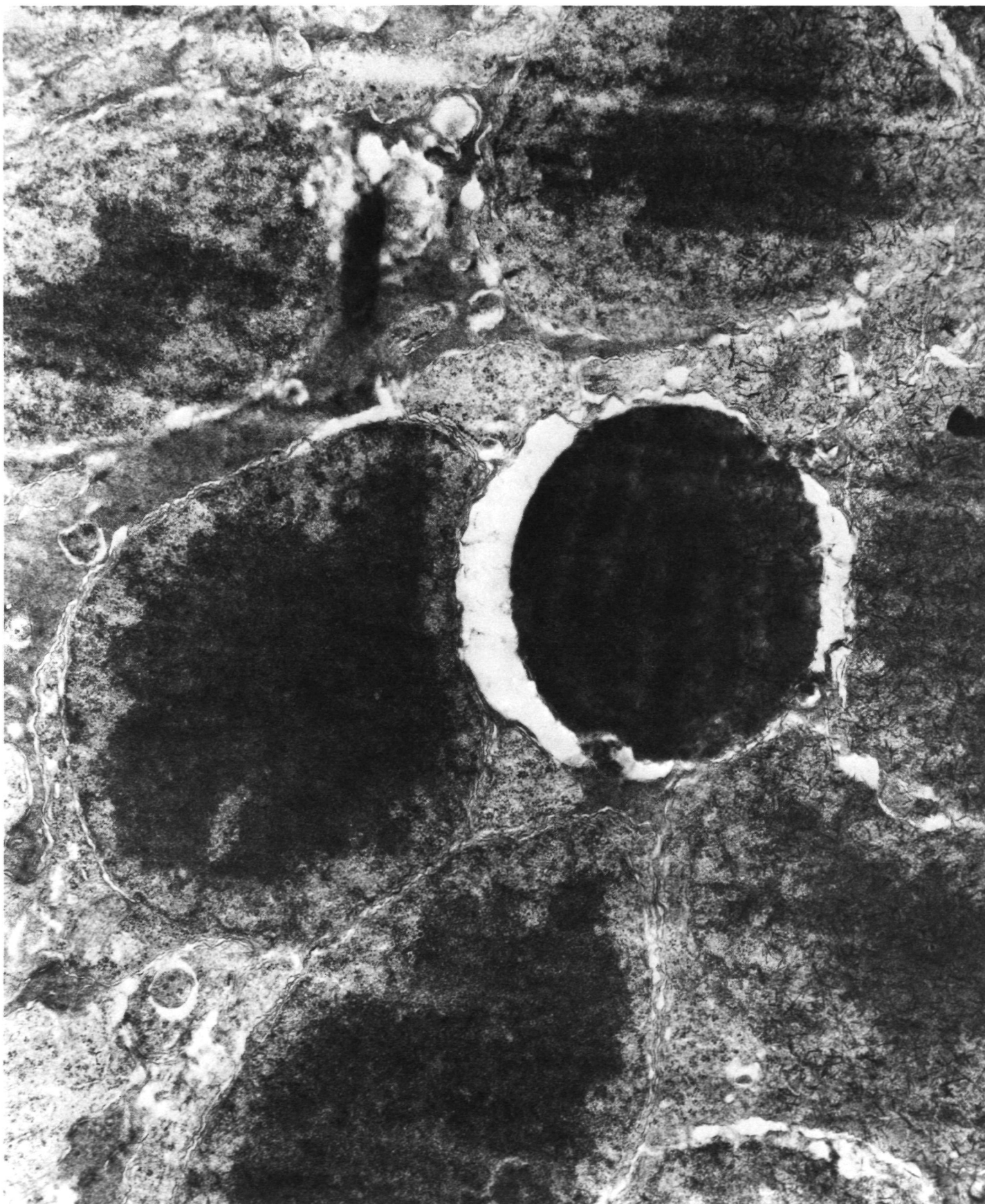


Fig. 3. Flight eye 25 days after recovery. Note the dark condensed nucleus and compare with that of Fig. 1. 19,500 X.



Fig. 4. Rare necrotic cell in the inner nuclear layer; 25-day recovery. 8250 X.

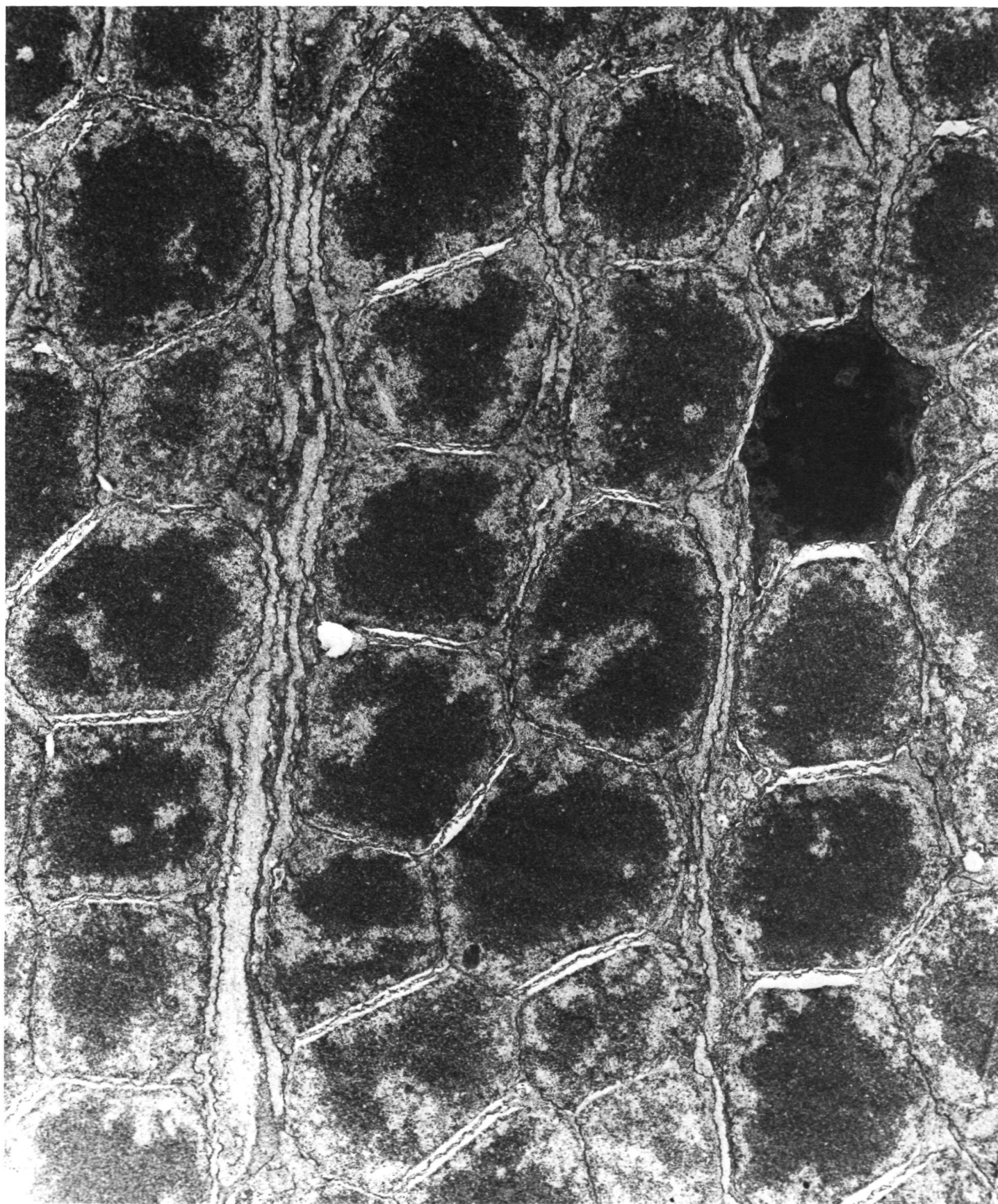


Fig. 5. Part of a 10-photograph composite from a flight plus zero
days eye. Note single dark nuclei in outer nuclear layer. 5500 X.
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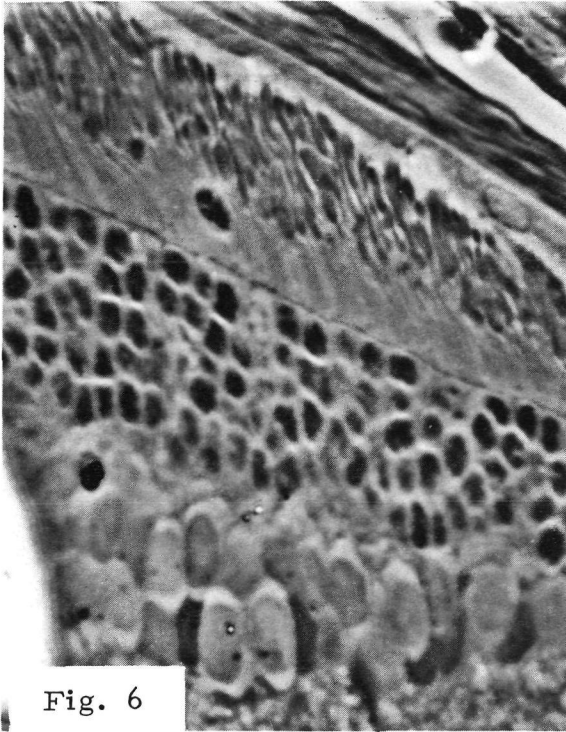


Fig. 6

Flight eye sacrificed at recovery. Note the displaced cell from the outer nuclear layer in the inner segment area, phase contrast. 950x

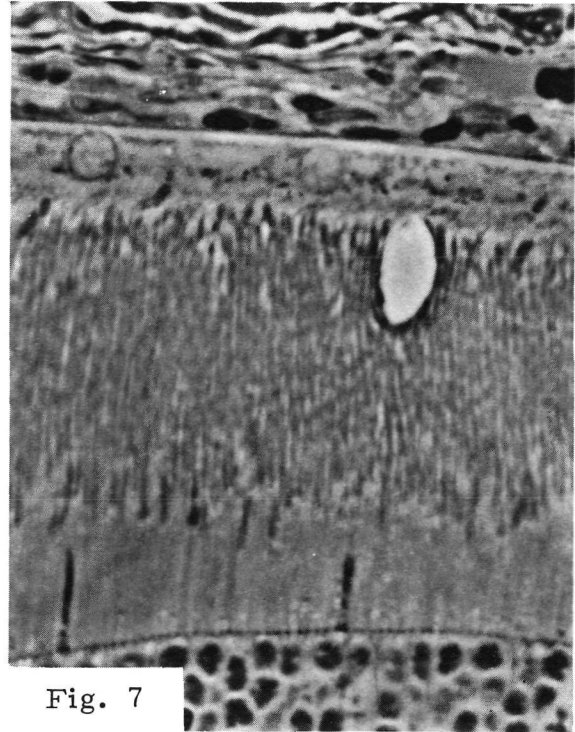


Fig. 7

Flight eye sacrificed at recovery. Part of a series of sections with a channel 10 μm long in the outer rod area, phase contrast. 950x



Fig. 8

Flight eye sacrificed at recovery. Large vesicle represents a channel 14 μm long in the outer rod area, phase contrast. 950x

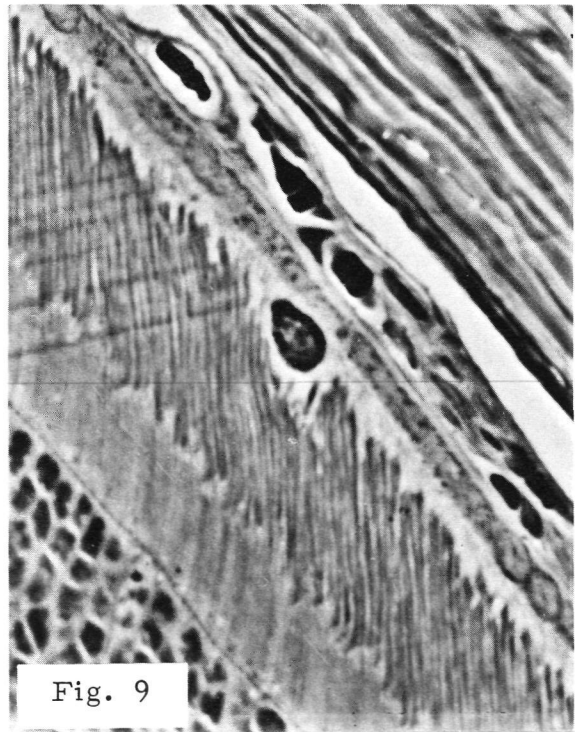


Fig. 9

Flight eye at day zero. A channel that goes for 26 μm has what appears to be a macrophage in this section. The channel runs between the rods and pigment epithelium, phase contrast. 950x

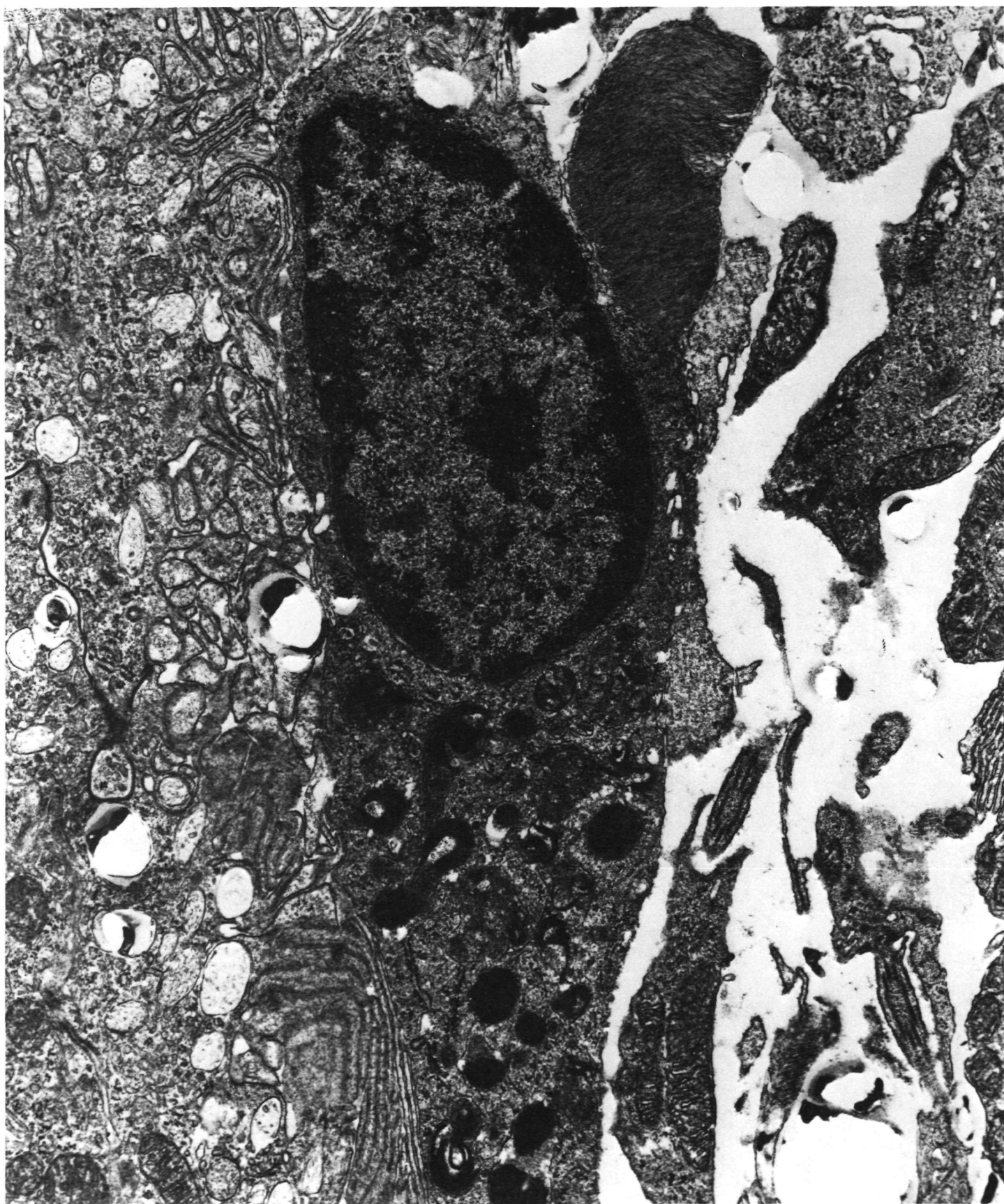


Fig. 10. Flight eye sacrificed 0 days after recovery. The macrophage is located between the pigment epithelium and the outer segments. 15,750 X.

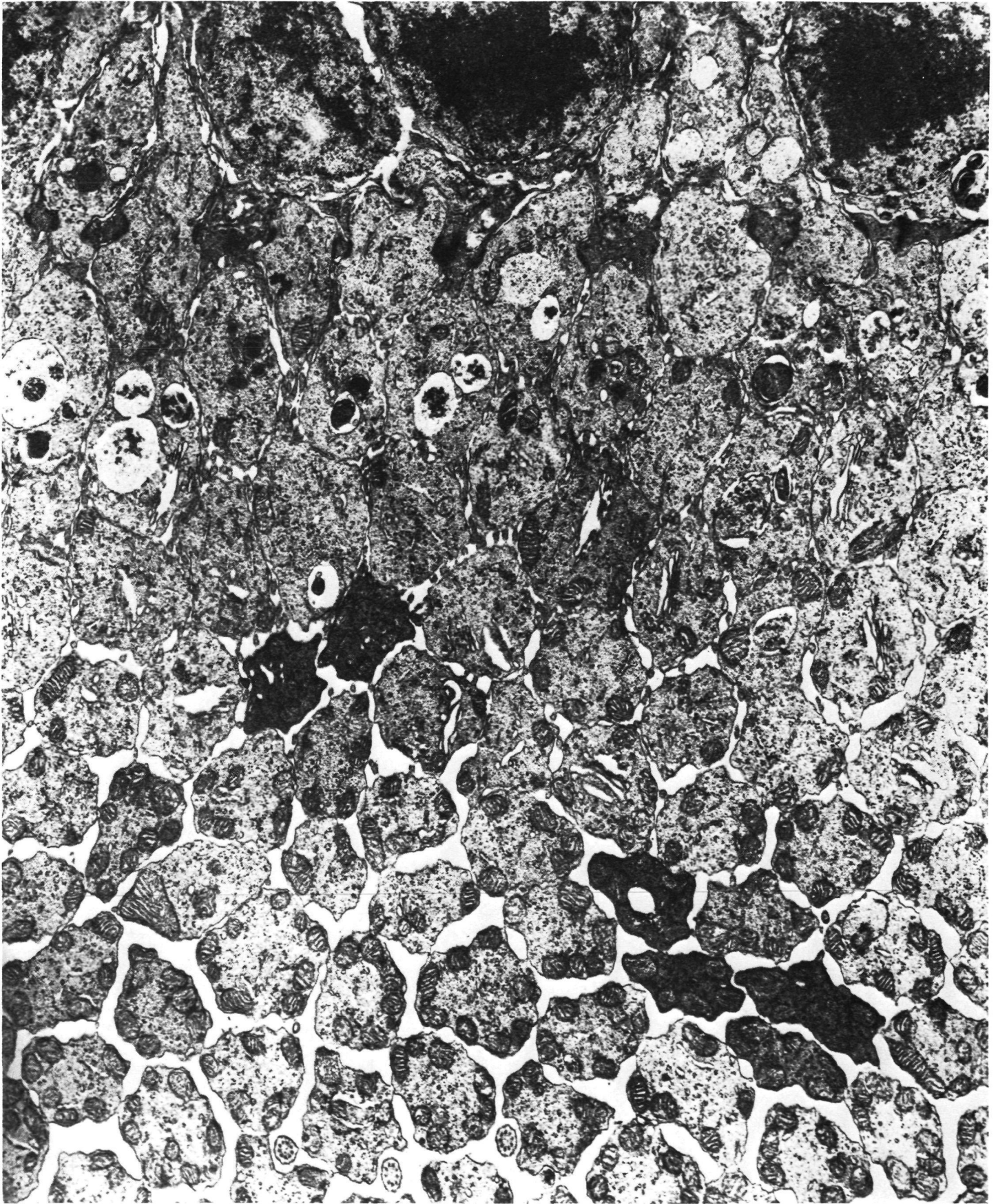


Fig. 11. Zero recovery time from flight. Vesicle in an inner segment which stains darker than most of the inner segments. 10,000 X.

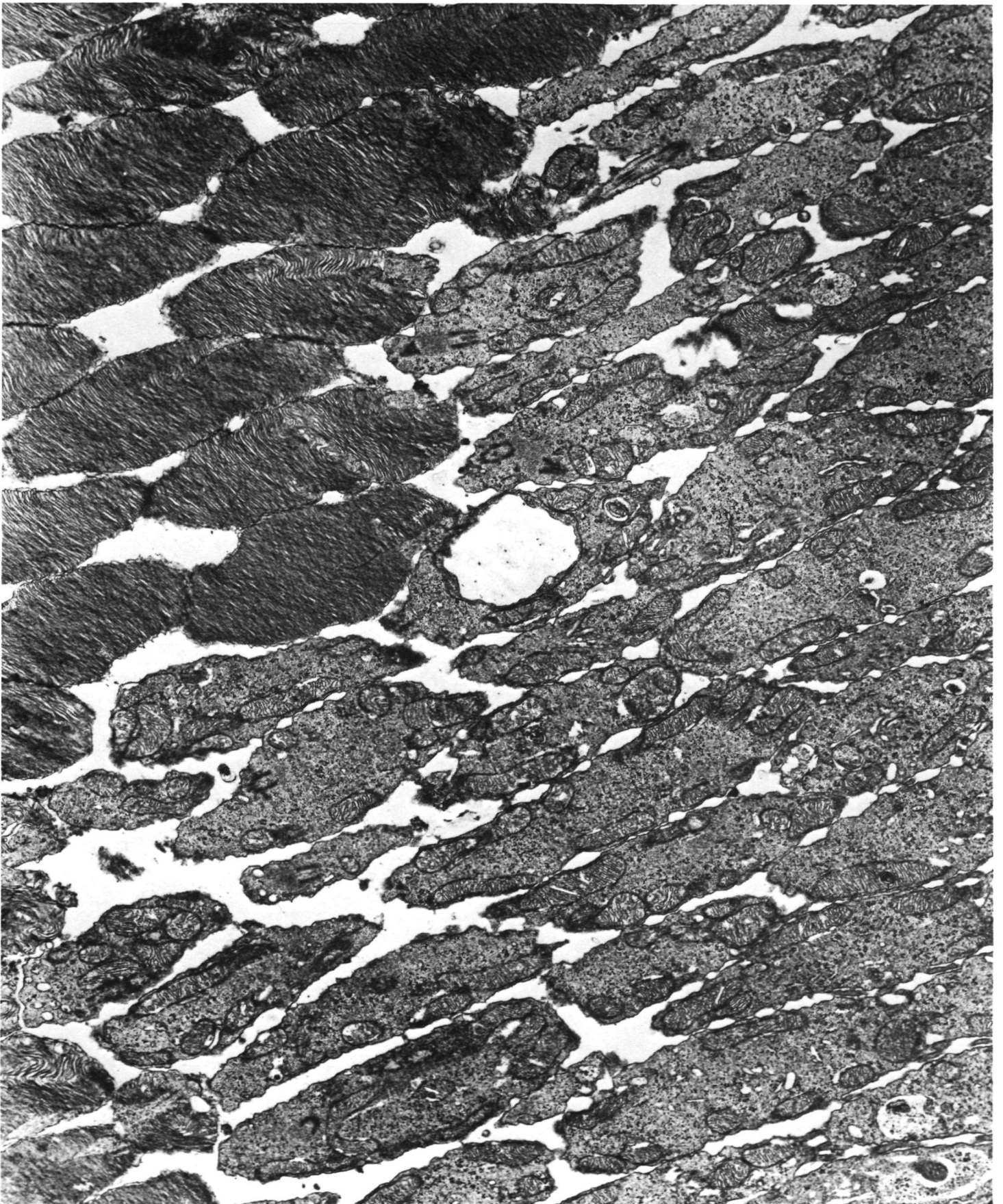


Fig. 12. Twenty-five days post recovery from flight. Note the large vesicle in the inner segment. 10,000 X.

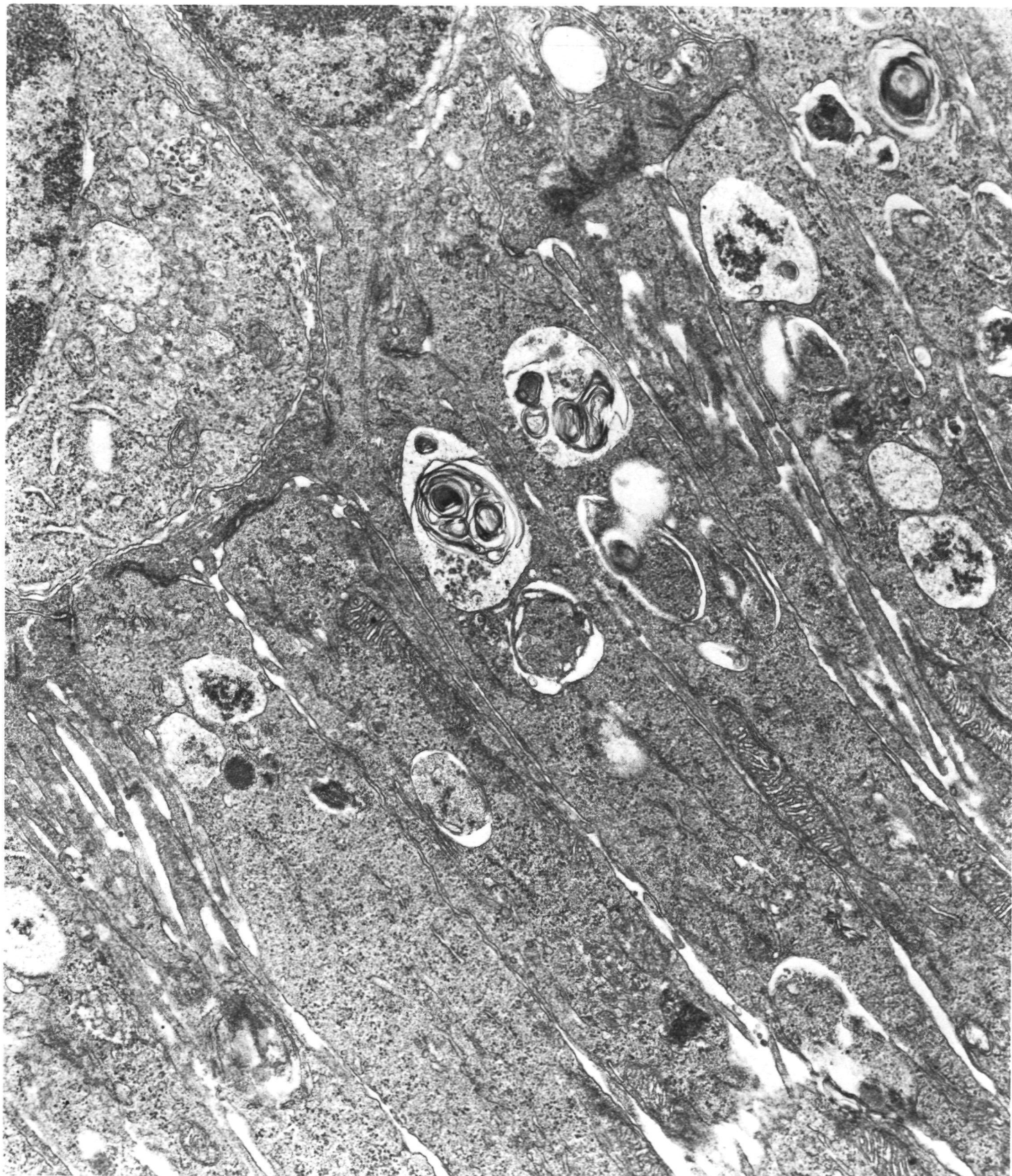


Fig. 13. Flight eye sacrificed at recovery containing an abnormal inner segment area. Note membrane pools and cellular breakdown. 19,500 X.

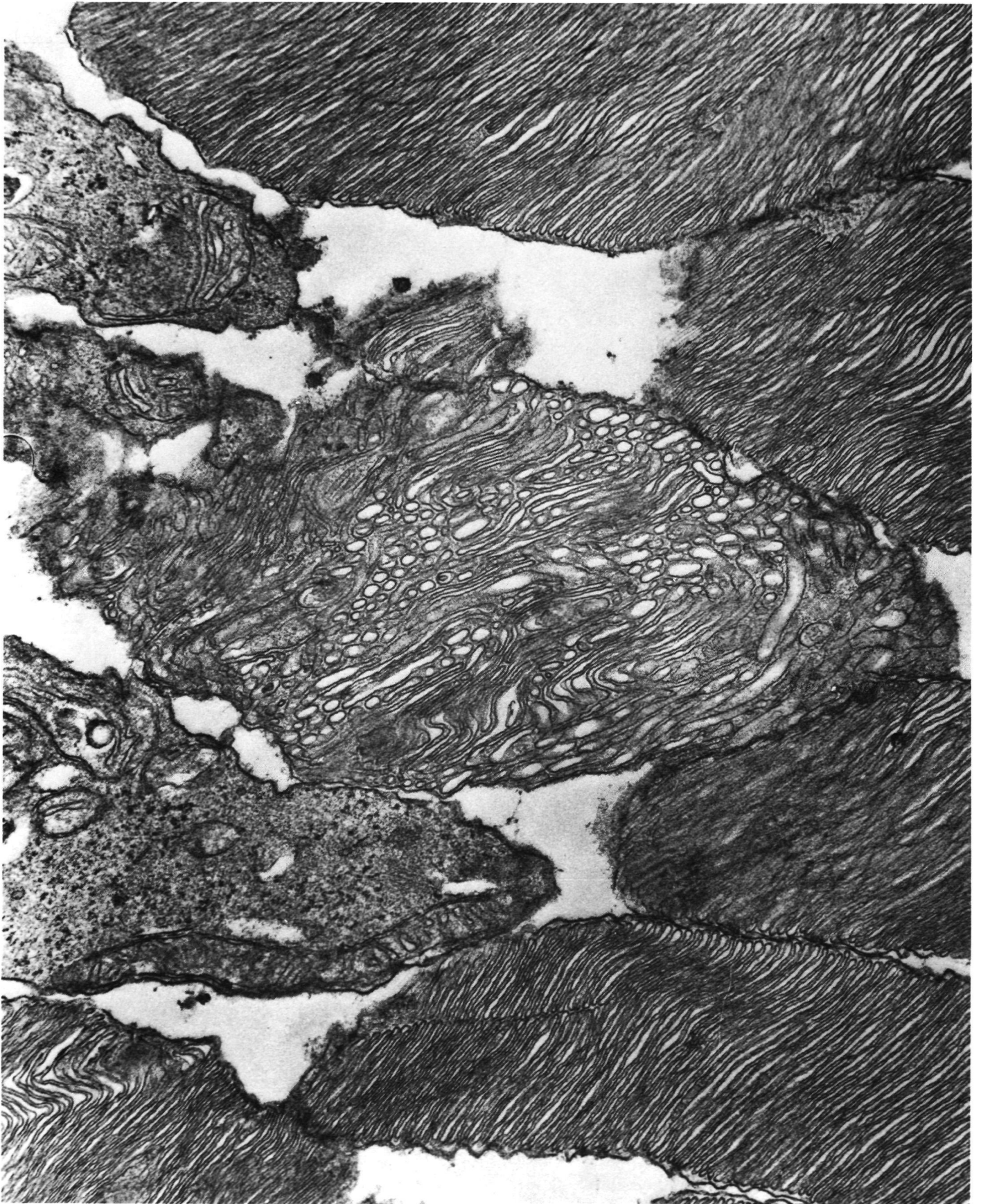


Fig. 14. Rod membrane disruption surrounded by normal ultrastructure;
25-day recovery. 42,500 X.



Fig. 15. Flight eye sacrificed 25 days after recovery. Note abnormal occurring pool of glycogen in the inner segment. 15,750 X.

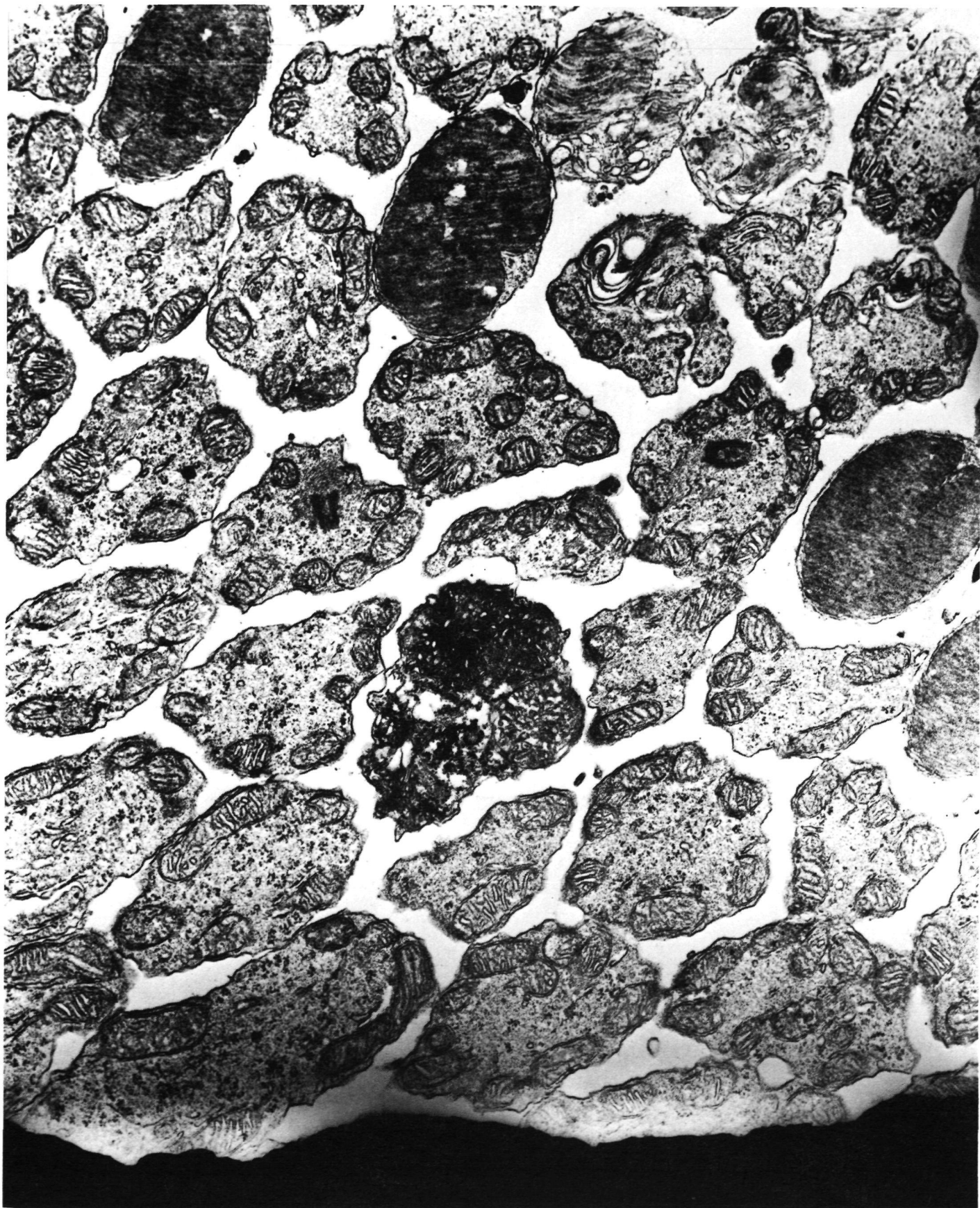


Fig. 16. Zero recovery time; centrally located inner segment has abnormal mitochondria and glycogen deposit. 19,500 X.

in areas of good preservation (Fig. 14). Rare but occasional pools of glycogen were seen in the inner segments (Figs. 15, 16).

The control eyes revealed some of the alterations seen in the flight specimens. Rod disruption was common. Two short channels were seen in the rod area but macrophages were not present. Occasional inner segments had debris, membrane whorls, and disrupted mitochondria.

The pigment epithelium showed varying degrees of outer segment ingestion and vesicles. One flight eye sacrificed on day zero contained a very large channel which was 28 μm long (Fig. 17). The outer plexiform layer was generally normal but one flight eye had a large swollen channel area almost filled with particulate material (Fig. 18). Most of the retina areas were normal in appearance (Fig. 19).

Discussion

The method for observing the eye tissue was modified somewhat from the routine electron microscopy to allow the large number of eyes, 72, to be examined. All of the material was embedded in plastic to allow sectioning of any area for electron microscopy. Even though 2- μm sections provided material easily observable by phase contrast microscopy, the silver stained specimens provided the best resolution for light microscopy. Since the alterations seen after irradiation at Berkeley could be observed in thicker than normal, electron microscopic sections, many were cut thicker, approximately 190 nm, to allow observation of a greater volume of tissue. The 2- μm sections for light microscopy can be lifted off the glass and viewed in a high

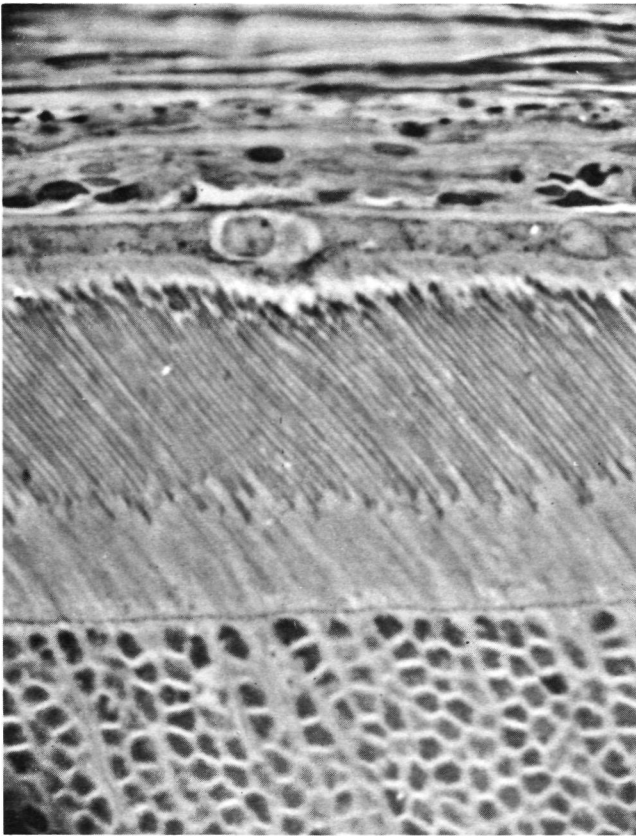


Fig. 17. Flight eye, zero recovery. Cell and debris in channel, 28 μm long located in the pigment epithelium, phase contrast. 950x

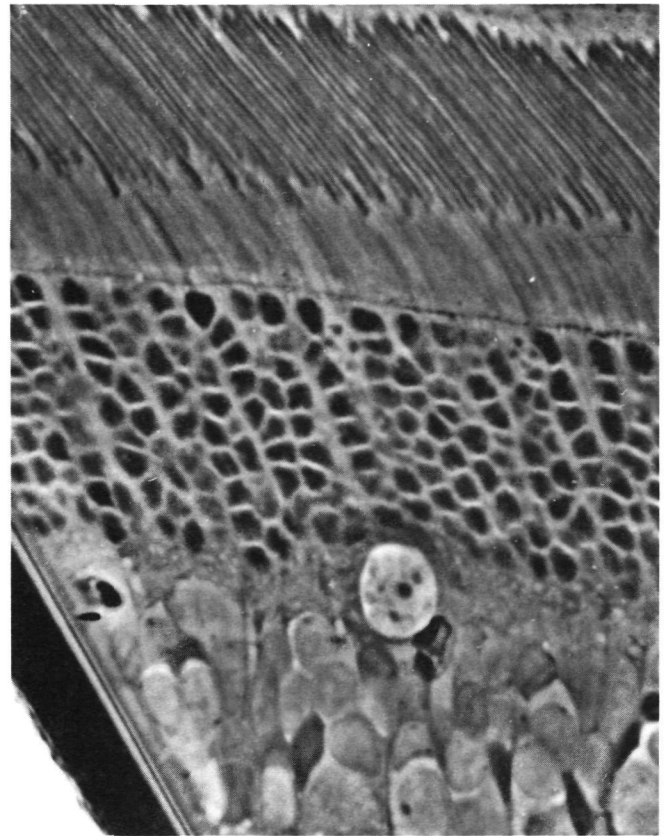


Fig. 18. Flight eye, zero recovery. Debris in channel 12 μm long in outer plexiform layer, phase contrast. 950x

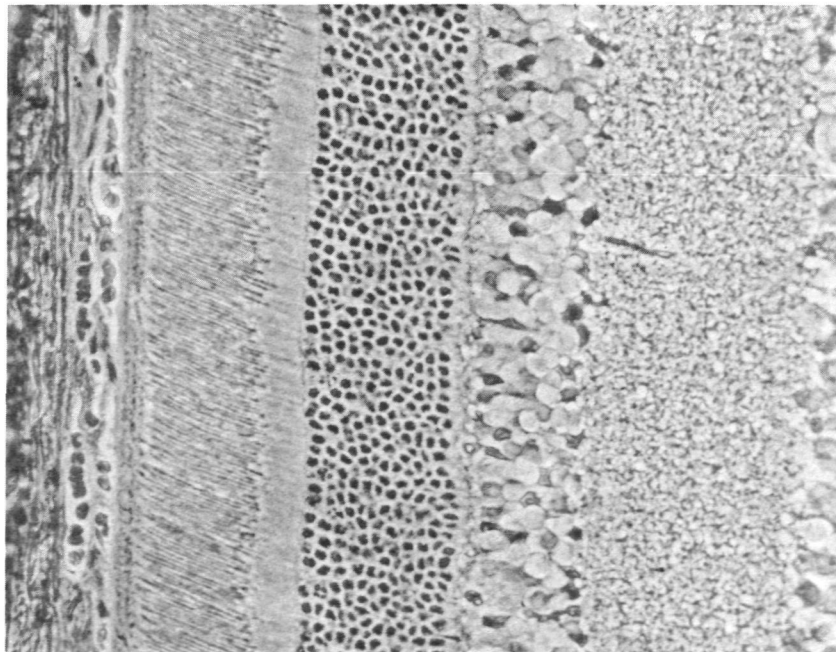


Fig. 19. Normal appearing retina from 25 days post recovery, phase contrast, silver stained. 250x.

voltage microscope. This phase of the microscopy will be done at a later date when time permits. To further increase the areas of tissue studied, large mesh or single-hole grids were used.

Evaluation of the fixation was achieved mainly by comparing the flight eyes to the controls. In addition, because only an estimated 123 particles were expected to enter each eye, most of the flight retinal tissue would therefore be expected to be normal. Any altered areas in the flight material could be compared to adjacent normal areas to insure that fixation had been adequate.

The clear channels were a surprising finding in the neon, argon, and K-007 flight exposures. Subsequent searching has turned up two short channels about the size of a cell in the controls. The reason for these channels is not understood. The 1000-rad exposures had about 10 hits per cell. Thus the areas with channels probably had particles traversing them. However, all the cells should have been hit and yet only a few channels were seen. The rods that had been exposed to nitrogen and oxygen beams also had enlarged clear areas; these were thought to have been caused by fluid moving into areas of molecular breakdown attributed to the radiation. Perhaps fluid also moved into these channel areas in response to osmotic demand. Indeed, debris could be seen in several channels but mostly they appeared clear; perhaps this was a consequence of the time of sacrifice.

So far these channels have been studied by light microscopy but the sections will also be observed in a high-voltage electron microscope. The presence of macrophages argues for cellular "cleanup" in these areas. Most of these channels occurred in the outer segment area which has been shown to be the best indicator of radiation damage

in the eye. If natural and radiation cell death could be occurring, the holes in the controls could have come from an occasional dying cell and the longer channels in the experimentals from radiation. However, the flight animals were not monitored and, therefore, any proof of particle traversal is lacking. Mechanical handling during preparation as a cause is a possibility; however, the neon and argon irradiated eyes contained channels and yet they had been fixed by perfusion of the animals through the heart without mechanical manipulation of the eye. This interesting observation will probably remain unresolved until proper tracking of the particles is carried out in the manner perfected for the Biocore experiment.

The inner segments of the flight eyes sporadically contained membrane debris, clear areas, and some small particulate material. Careful examination of the controls also revealed areas similar to those in the flight eyes. Presently it appears the flight eyes may show slightly more of this type of change but the difference is not regarded as significant.

The blood vessels of the flight and controls appeared normal. However, Fundus photography could not be done. In view of the demonstration of the sensitivity of monkey retinal blood vessels to nitrogen and oxygen particles (1) this sensitive parameter should be measured after the next flight. The capillaries in the choriocapillaries contained a number of platelets. This was true of the neon, argon, and flight specimens but the controls appeared to have fewer. The blood vessels are known to become permeable shortly after irradiation and the increase in the number of platelets might indicate that change in permeability.

Cells of the outer nuclear layer responded to 1000 rad of argon and neon with sporadic cytoplasmic swelling, clearing, and cell death. In comparison, fewer enlarged and necrotic cells were seen in the flight eyes than were seen in those of animals irradiated with argon and neon. The cells were distributed in a random fashion.

The displacement of the outer nuclear layer nuclei into the inner and outer segment area was a surprising finding in the Berkeley exposures and one such displacement has been seen in a flight (F+0) eye. While again the answer is not clear, two possible explanations may exist. If radiation weakens the tight junctions which form the outer limiting membrane then these cells could slip through more easily. Normally, these junctions provide mechanical support to hold the retina together. If the outer limiting membrane is not weakened, then mechanical pull could be a possible cause of displacement. Comparison of the hundreds of sections for light and electron microscopy gave at least the subjective impression of fewer alterations in the 25-day postflight animals compared to those sacrificed immediately after recovery. This was unexpected, since the 25-day postflight period was thought to allow some time for possible lesions to develop. However, many of the observed symptoms (see other reports such as muscle, kidney, etc.) including muscle coordination returned to normal in the 25-day period. Perhaps there was some recovery of the eyes as well. The same fixation techniques and solutions were used for both preparations.

Damage to the outer rod segments in the experiment with nitrogen and oxygen seems to implicate the rod cells as the particularly sensitive elements in the retina. Indeed, in our experience the outer

nuclear layer has been the most responsive to radiation from HZE particles. One would probably expect this because the rod cell nuclei are so densely packed in the outer nuclear layer that a traversing particle would pass through a minimum of five or six nuclei if traveling at right angles across the retina. More nuclei would be hit if the trajectory were more horizontal.

Since rod cells are highly specialized, fewer transcriptional events are probably occurring in the nucleus. This would seem in turn to narrow the target area in which a particle could cause significant damage to the nucleus or some sensitive part of it. Even though the DNA damage from irradiation with HZE particles has been shown to undergo "repair" (Lett, J., unpublished), the fidelity of this repair is not known. Nuclear damage could result in cell death or in transcriptional or translational defects and, because rod cells only renew themselves by renewal of cell constituents, disrupted activity could lead to ultrastructural changes visible in the electron microscope. Indeed, this is the present finding and scanning microscopy also shows rod disruption in Lawrence Berkeley exposures. Thus, the dense packing of the nuclei makes this layer most useful for radiation studies and it has furthermore been consistently the most responsive one to radiation from HZE particles at Berkeley. Perhaps refinement of dose response in this layer could provide a biological dosimeter. Present evidence would induce us to believe that the light flashes are produced when a HZE particle traverses the outer segments. Damage, when it occurs, would seem to arise from the particles hitting a critical area of the nucleus. However, membrane alterations in the rods could also be a

direct result of HZE interaction. The mechanism for HZE interaction with the tissues is not as yet really understood. The retina, because of its unique structural arrangement, seems quite appropriate for this type of study.

Acknowledgment

The assistance of Debra Nagao and Katharine Kato for preparing sections, slides and prints for this work is greatly appreciated.

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EFFECTS OF WEIGHTLESSNESS ON THE EMBRYONIC DEVELOPMENT AND
AGING OF DROSOPHILA

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A B S T R A C T

The biological effects of weightlessness have been investigated on Drosophila melanogaster of the Domodedov-32 strain, which developed and spent the first days of adult life in space. Following a 19.5 day exposure to zero g, the flies were studied by morphological, chemical and behavioral techniques. Apparently, the development of Drosophila was insensitive to weightlessness and the aging process was not influenced, except for a slight reduction in the amount of lipofuscin present in the midgut and Malpighian tubules.

KEY WORDS: Weightlessness, Drosophila, Embryonic development, Aging.

INTRODUCTION

All living organisms have evolved under the influence of the gravitational field of the earth, and, therefore, it is generally assumed that gravity has played a role in shaping their structure and function (2). This, however, could not be experimentally confirmed until the advent of the Space Age. Even a biologist of the scientific stature of Darwin, commented that we would never be able to escape gravity and perforce would remain ignorant concerning its role in evolution and development (14). About one hundred years after Darwin, the space programs of the USSR and the USA have made space travel possible and numerous observations have documented the important physiological effects of weightlessness on human subjects (12, 13, 20). On the other hand, space flight experiments suggest that zero g does not influence in a drastic manner fundamental biological processes such as embryogenesis and growth, at least as regards insects. In effect, the Tribolium experiment performed as a part of the Biosatellite II program failed to demonstrate any effect of weightlessness on the development of this insect (1). This is in agreement with numerous observations performed on another insect species, namely Drosophila melanogaster, which has been exposed to zero g in Soviet manned vehicles and unmanned biosatellites. As stated in a review of USSR space biology, published in 1968 (24): "Copulation, oviposition and normal development of Drosophila can occur normally during periods of weightlessness up to four days. Embryonic development of a normal strain of Drosophila under weightless conditions does not result in statistically significant increases in morphological changes or mutations in sex cells. In these experiments on "Vostoks 3 and 4", however, the number of females in all cultures considerably exceeded the number of males, and the latter hatched somewhat latter".

More recently, the Soviet space biologists showed that Drosophila exposed to weightlessness during a 22-day "Kosmos" flight could complete nearly two entire developmental cycles from fertilization to hatching. Therefore, the

conclusion was reached that "...weightlessness or even any other factors of space flight could not have affected the duration of Drosophila development" (19).

Previous work from our laboratory has documented that the aging process of Drosophila is strikingly sensitive to altered environments, including temperature changes (16) and rotation in a clinostat (16). Moreover, data from Biosatellite II show an apparent increase in the life span of Habrobracon wasps which were exposed to zero g (22). This, in our opinion, made advisable to extend the research on the effects of weightlessness to the last stages of the insect life cycle, i. e. the adult period, including senescence. Therefore, the present work focuses in the aging process of fruit flies, which developed and spent their first days of adult life in a "Cosmos" biosatellite.

METHODS

The flies used in this study were wild Drosophila melanogaster of the Domodedov-32 strain. They were placed in the Soviet biosatellite as larval cultures, in vials containing standard medium. At launch, the flies were 4 days old, as estimated from oviposition time. The experimental Drosophila population was exposed to weightlessness during about 19.5 days, and an equal number of flies, which were housed in a centrifuge at approximately 1 g aboard the "Cosmos" vehicle, served as flight controls. Two other groups, namely "synchronous" and "laboratory" controls were part of the experiment. The synchronous flies were kept in a mock-up of the biosatellite, on the ground, throughout the time of the space flight. The laboratory controls were Drosophila maintained on the ground under standard laboratory conditions.

During space flight in the biosatellite the flies were exposed to the following environmental conditions: 742-850 mm Hg of total atmospheric pressure; $pO_2 = 140-253$ mm Hg; $pCO_2 = 2.3-8.0$ mm Hg; temperature = 19-21.3°C; relative humidity = 40-62 %. The flies were maintained in total darkness for the entire duration of the flight.

Additional data on the experimental plan and flight factors can be found in the introductory article on the "Cosmos-75" flight.

Table 1 shows the number of Drosophila which were delivered to the Ames laboratory in satisfactory condition, at 28-29 days after launch and 7-8 days after landing of the biosatellite. Total exposure of the experimental Drosophila to weightlessness was approximately 19.5 days. Since hatching was expected to occur on the 7 or 8th day after launch, adult Drosophila were only exposed to weightlessness during a period of 12 to 13 days.

Upon arrival at the Ames laboratory all wild flies were individually weighed in a Cahn electric balance. Then, they were transferred to vials containing fresh food and placed in incubators at 21°C, under continuous light of moderate intensity. Humidity was approximately 40 %. Transfer to fresh food vials was performed once a week, as in previous work from our laboratory (3,4,6,7,8,10,15).

The following parameters were investigated on experimental and control flies:

- 1) Body weight, which was determined on all flies, following etherization.
- 2) Vitality, as expressed by negative geotaxis and mating. Negative geotaxis is measured using a glass-stoppered volumetric cylinder of 250 ml fitted with a layer of soft plastic on its bottom. When shaken to the bottom by tapping the cylinder against a rubber mat, the flies quickly run or fly to the upper region. From 20 to 50 flies are used in each measurement, and the results are expressed as the percent of flies which cross the 250 ml mark in 20 seconds. Mating competence was determined as in previous research (4). Individual males were placed in empty vials (15 cm in length and 2.5 cm in diameter). Then, three 7-day-old virgin females were placed in each tube,

Table 1: Number of experimental and control wild Drosophila melanogaster of the Domodedov-32 strain used in this investigation.

	<u>♂ Number</u>	<u>♀ Number</u>
Experimental (exposed to weightlessness)	101	100
Flight controls (maintained in a centrifuge at ~1g)	91	97
Synchronous ground controls	52	46
Laboratory ground controls	43	48

a cotton plug was pushed half-way into the tube and the mating behavior of the flies was observed for one hour. The time to the first mating (mating latency) and the number of matings in one hour were recorded for each of the male flies tested. At the end of one hour, males and females were separated and saved for future studies, including quantitative determination of the offspring produced.

- 3) External morphology, as demonstrated by gross photography and scanning electron microscopy of control and experimental flies.
- 4) Age-associated degenerative changes, investigated by light and electron microscopy using the techniques described in our previous publications (6,7). Ten flies from each group were used for these morphological studies.
- 5) Glycogen content of the thorax. This parameter was investigated by freezing the flies in liquid nitrogen, separating the thorax from the head and the abdomen, and analyzing the glycogen by the glucose oxidase technique, following extraction with KOH and ethanol precipitation. Since the Drosophila thorax is packed with wing muscles, the glycogen analysis provides a good estimate of the energy reserve of the muscles which support the insect flight.
- 6) Life span, was determined in a sample of each group of male and female space-flown Drosophila. Since the time of hatching was not known with exactitude, all ages in the present article are given in days since the laying of the eggs from which the imagoes used originated.

RESULTS

The mean body weights for all Drosophila populations, at 33 days of age, are shown in Table 2.

Table 3 summarizes the results of the analysis of thorax glycogen and Tables 4 and 5 give the values obtained for negative geotaxis and mating competence of experimental and control flies.

Various aspects of the external morphology, which was normal for all Drosophila,

Table 2: Body weights of flies exposed to weightlessness and controls,
at 33 days of age.

<u>Males</u>		<u>Mean body weight (in mg) $\pm$ S. E.</u>	
<u>Number of</u>			
<u>flies</u>			
43	Laboratory controls	0.948	0.011
52	Synchronous controls	1.098	0.009
91	Space-flown at ~1g	1.060	0.012
101	Space-flown at ~zero g	1.067	0.009
<u>Females</u>			
48	Laboratory controls	1.354	0.016
46	Synchronous controls	1.527	0.023
97	Space-flown at ~1g	1.585	0.019
100	Space-flown at ~zero g	1.588	0.015

Note: The space-flown male Drosophila are lighter than synchronous controls and heavier than laboratory controls ($p = 0.030$ and $p < 0.001$, respectively).

Table 3: Glycogen content of the thorax of 48 day old flies. (The analysis was performed on samples containing two isolated thoraces).

<u>Number of samples</u>		<u>μg of glycogen per 2 thoraces: mean values ± S. E.</u>	
5	Synchronous controls	37.9	1.6
5	Space-flown at ~1 g	35.9	1.1
5	Space-flown at ~zero g	34.6	1.6

Table 4: Negative geotaxis of control and experimental male flies, at 65 days of age.

Percent of flies able to reach the 250 ml mark in a volumetric cylinder in 20 seconds (mean of 10 consecutive trials).

Synchronous controls	85
Space-flown at ~1 g	73
Space-flown at ~zero g	78

Table 5: Mating competence of male flies tested at 62 days of age. (Every male used in this experiment was kept in a test tube with 3 young virgin females of our control D. melanogaster Domodedov-32 population. The time to the first mating (latency) and the total number of matings in 1 hour were recorded for each male fly tested).

<u>Number of males</u>		<u>Total no. of first matings</u>	<u>Total no. of 2nd matings</u>	<u>Average no. matings/♂</u>	<u>Latency (min.)</u>
20	Synchronous controls	20	16	1.5	6.6
20	Space-flown at ~1 g	15	7	1.1	12.1
20	Space-flown at ~zero g	19	8	1.3	11.6

including the population exposed to weightlessness, are illustrated in Figures 1 to 8.

As shown by Figures 9 and 10, the fine structure of the wing muscle of flies exposed to space weightlessness was perfectly normal. Moreover, the fat body, oenocytes (Figure 11) and testes (Figure 12) could not be distinguished on the basis of their fine structure from the same organs of the flight controls.

As in old Drosophila melanogaster Oregon R from our laboratory (6,10,17), lipofuscin accumulation was very striking in the oenocytes of old D.melanogaster Domodedov-32, including the flies exposed to weightlessness (Figure 11). Moreover, the accumulations of intramitochondrial crystals and virus-like particles first observed by us in D. melanogaster Oregon R, were also noticed in the Domodedov strain (Figures 13 and 14).

Seemingly, the only fine structural difference between Drosophila exposed to weightlessness and the flight controls was a somewhat better preservation of the cells of the midgut and of the Malpighian tubules. The dense body accumulation characteristic of the cells of old Drosophila (Figures 15 and 16) was not so evident in the flies which were exposed to zero g. (Figure 11). However, this effect was not accompanied by an increased longevity, since as shown in Table 6, the life span of the Drosophila exposed to weightlessness was not significantly increased as compared to that of the control populations.

DISCUSSION

Our present observations are in agreement with previous research by Antipov and Parfenov (cited by Wukelic, 24) and with more recent data by Parfenov (cited by Dubinin and Vaulina, 2) showing that weightlessness does not seriously interfere with Drosophila development. An apparent lack of striking effects of zero g on the embryonic development of amphibians (25) and fish (21) has also been documented by American space biologists.

It is conceivable that prolonged exposure to weightlessness may induce wing

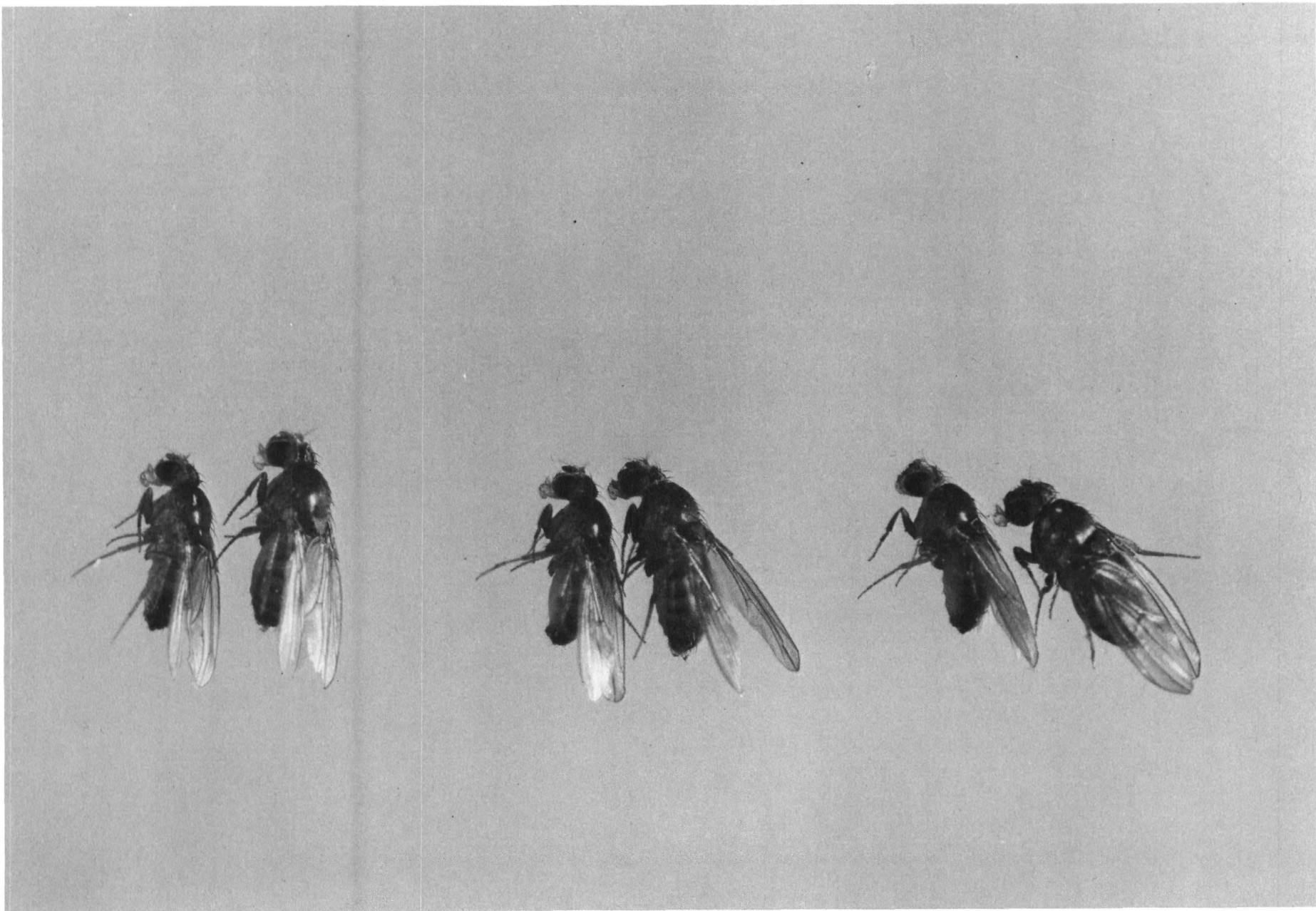


Fig. 1. Synchronous ground controls and space-flown Drosophila. Approximately X10. Right male and female couple: synchronous controls. Middle couple: flies which were exposed to weightlessness aboard the Cosmos biosatellite. Left couple: flight controls maintained in a centrifuge at about 1 g in the biosatellite.

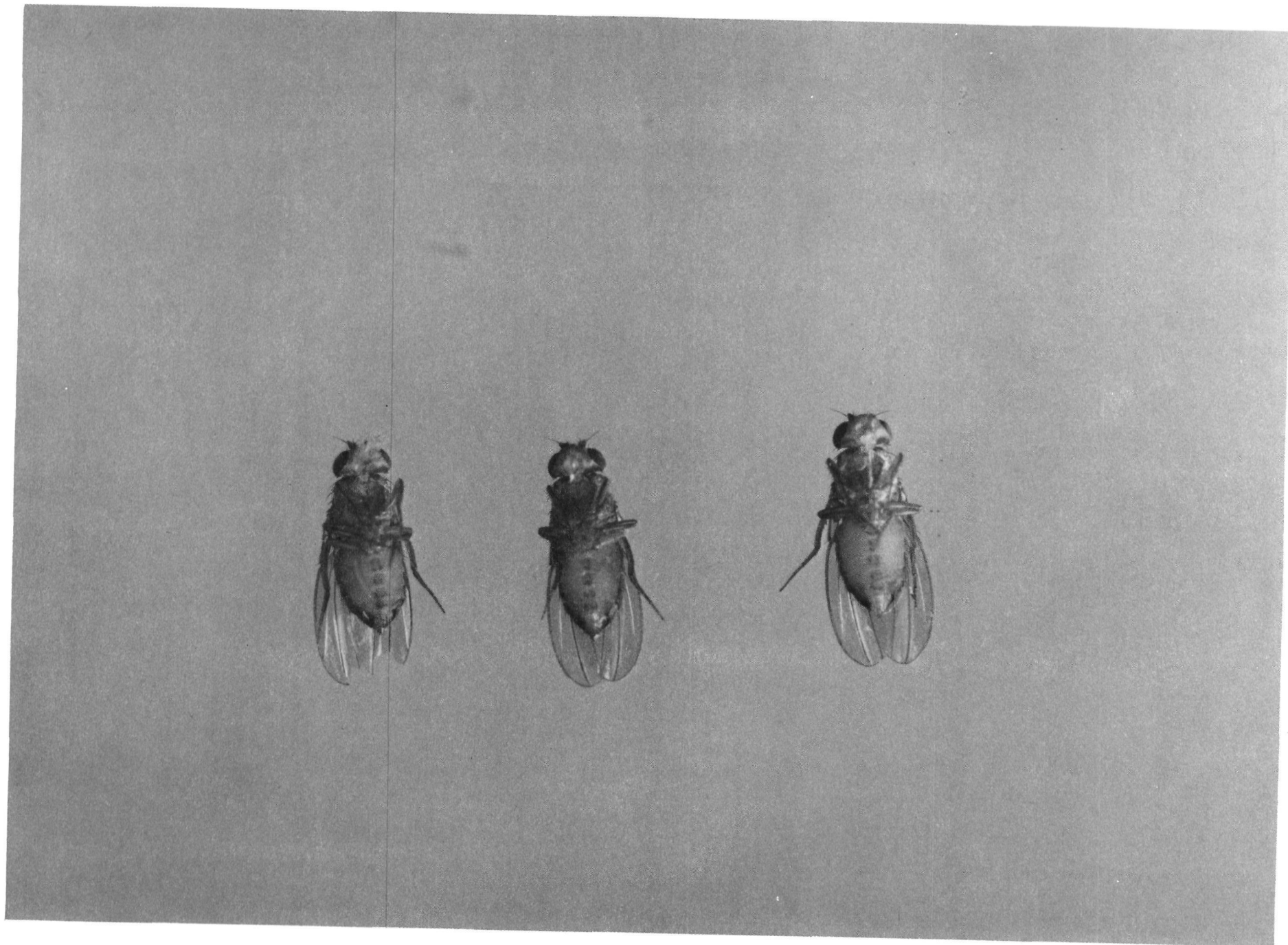


Fig. 2. Synchronous ground controls and space-flown Drosophila. Approximately X10. Ventral view of flies arrange in the same order as in Fig. 1.

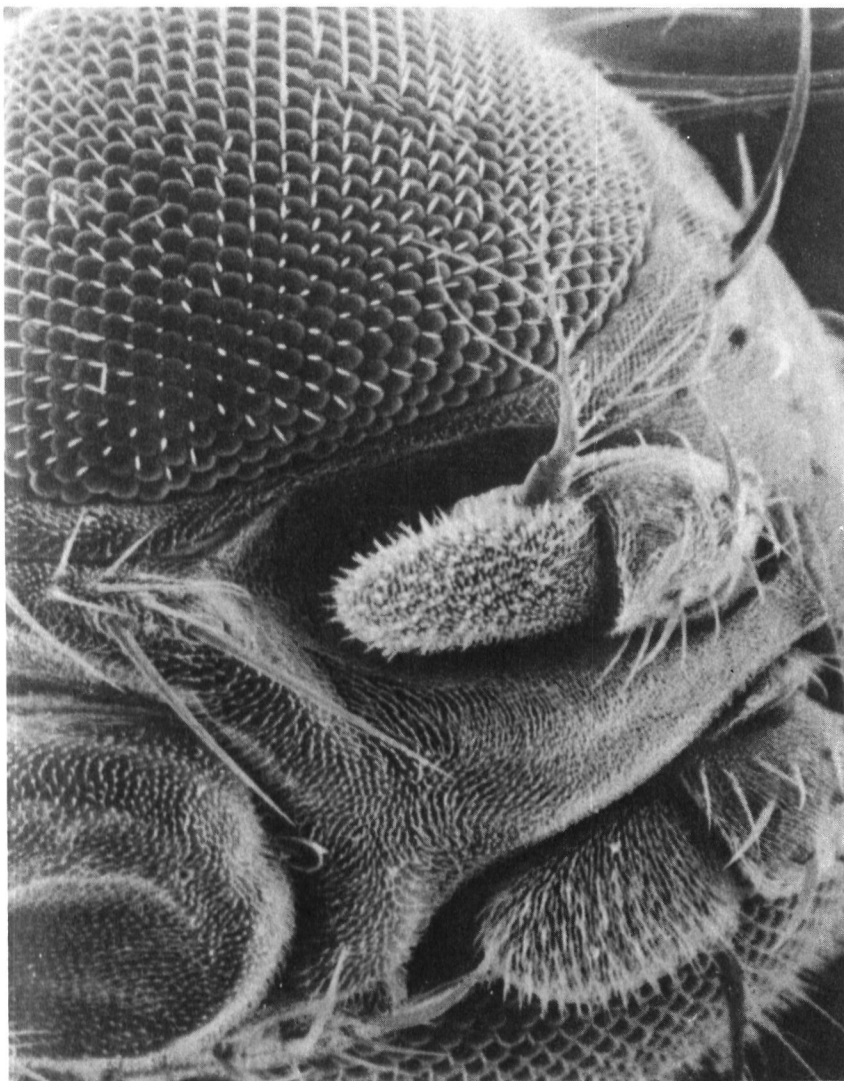


Fig. 4. Drosophila flown in a centrifuge aboard the biosatellite. External morphology of the head. X570.

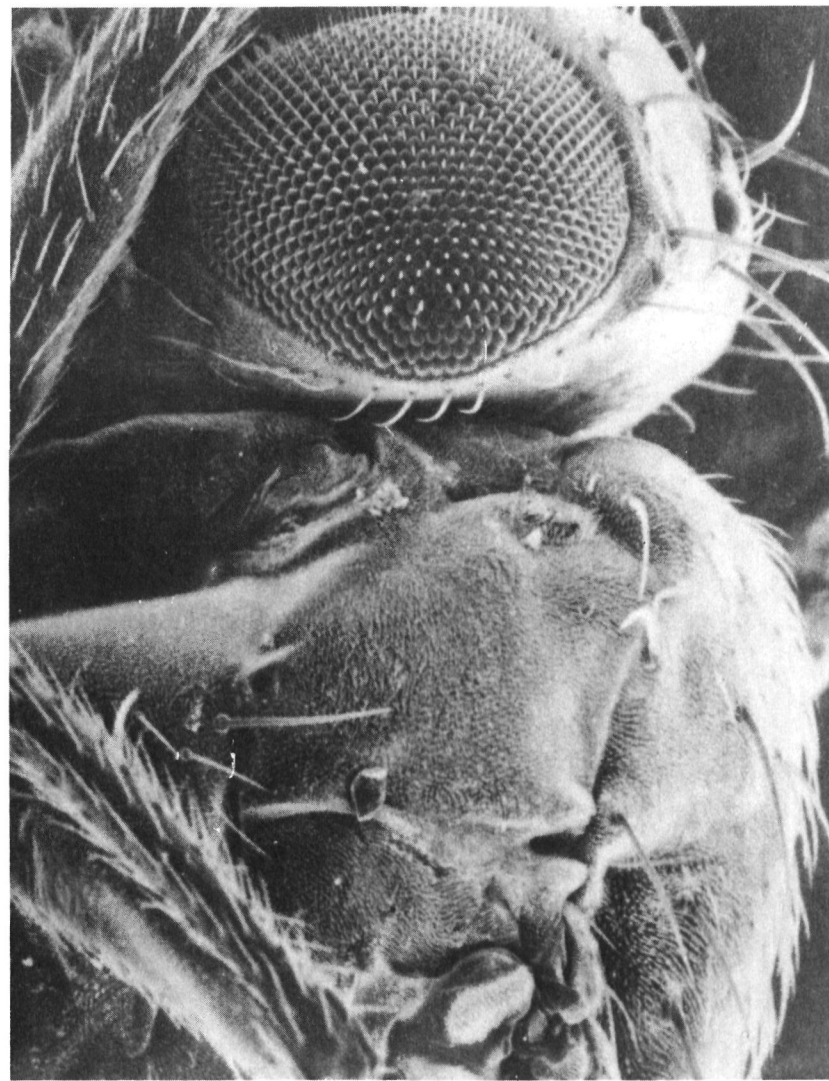


Fig. 3. Drosophila flown in a centrifuge aboard the biosatellite. Side view of the head and thorax. X390

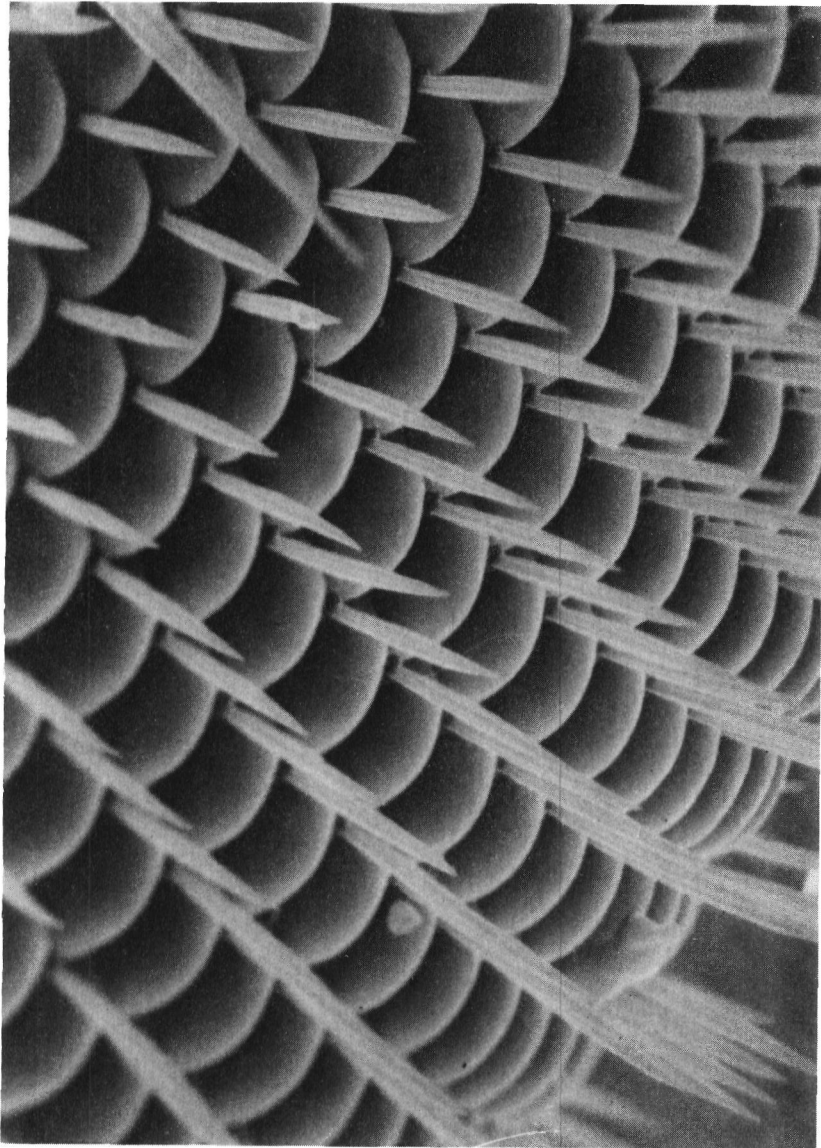


Fig. 6. Drosophila flown in a centrifuge aboard the biosatellite. High magnification scanning electron micrograph of the lenses and setae. X3000.

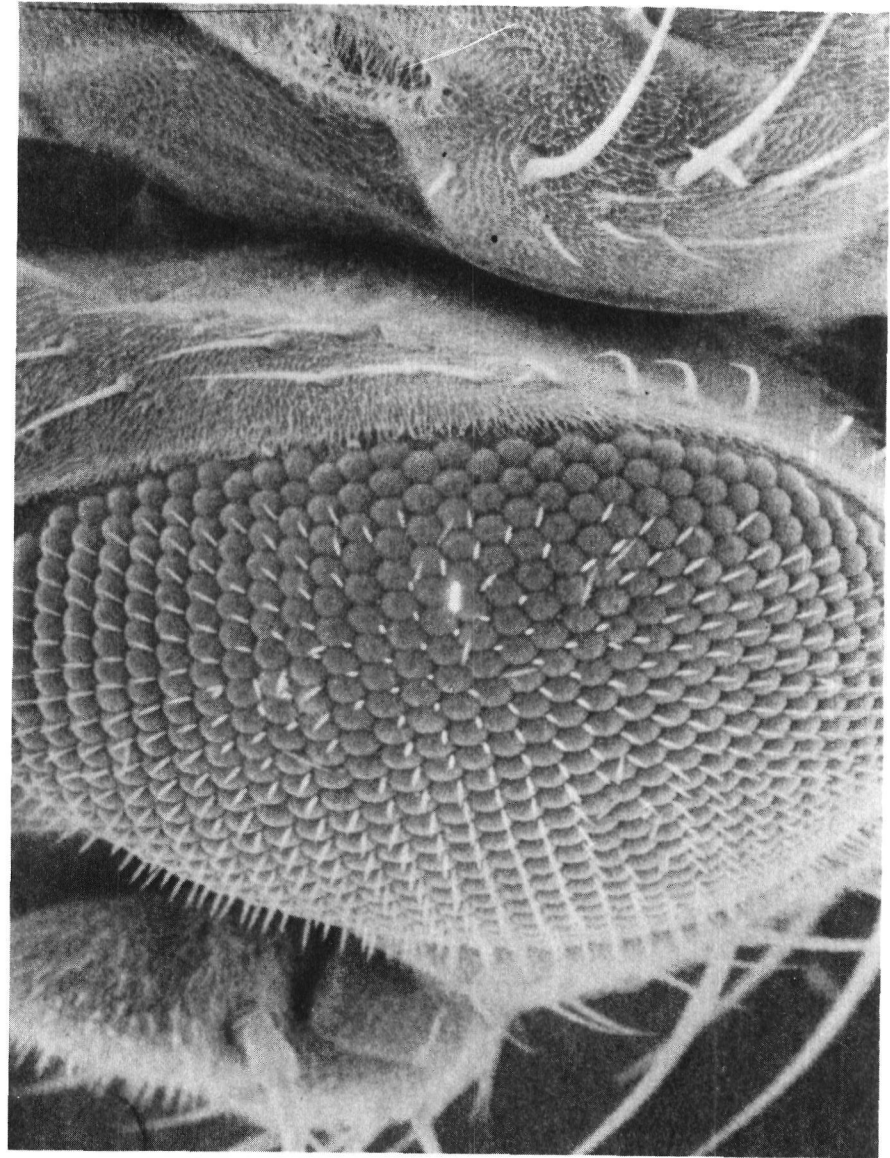


Fig. 5. Drosophila flown in a centrifuge aboard the biosatellite. Eye, showing the lenses and setae. X500.

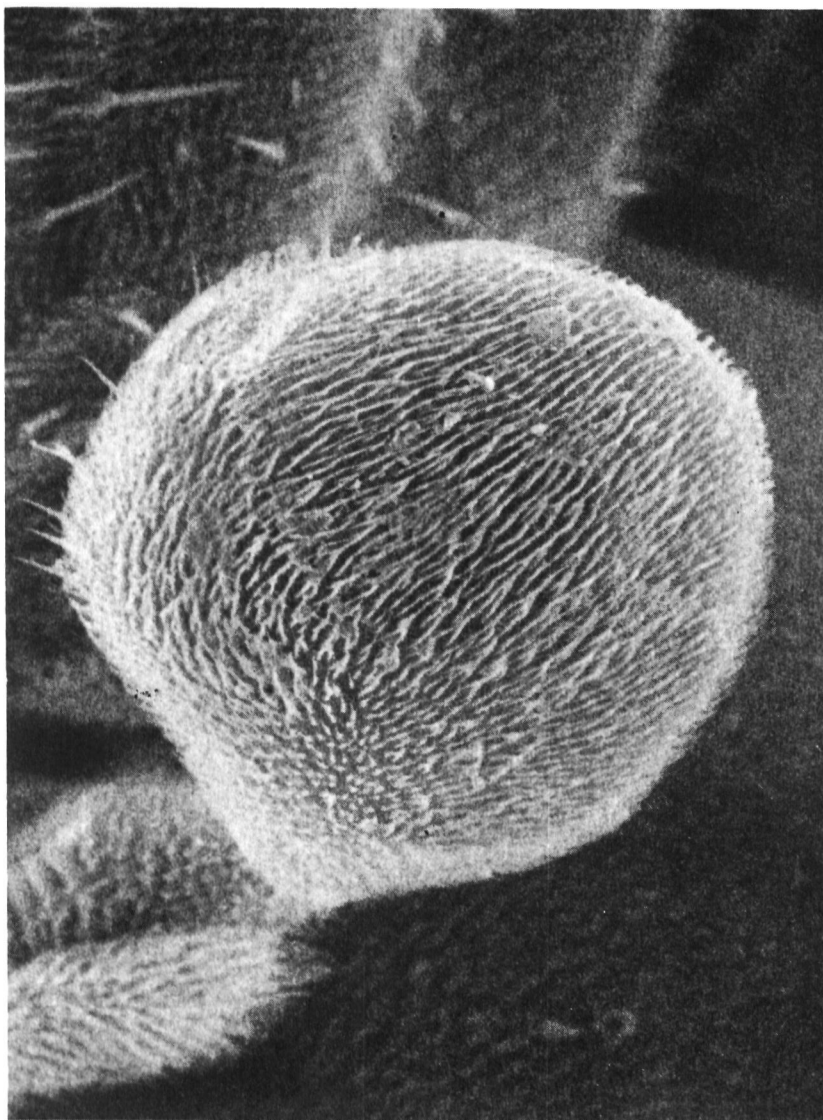


Fig. 8. Drosophila flown in a centrifuge aboard the biosatellite. Haltere (equilibrium organ). X500.

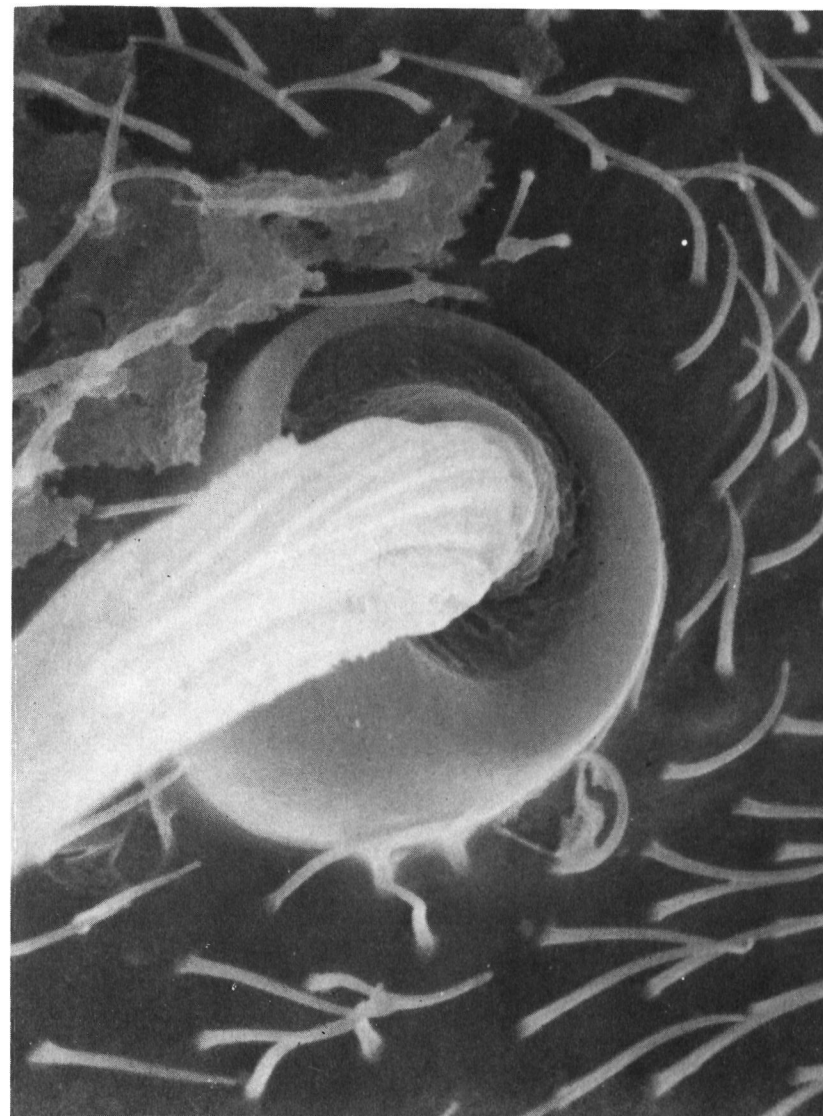


Fig. 7. Drosophila flown in a centrifuge aboard the biosatellite. Morphology of one of the vertical setae of the head. X2000.

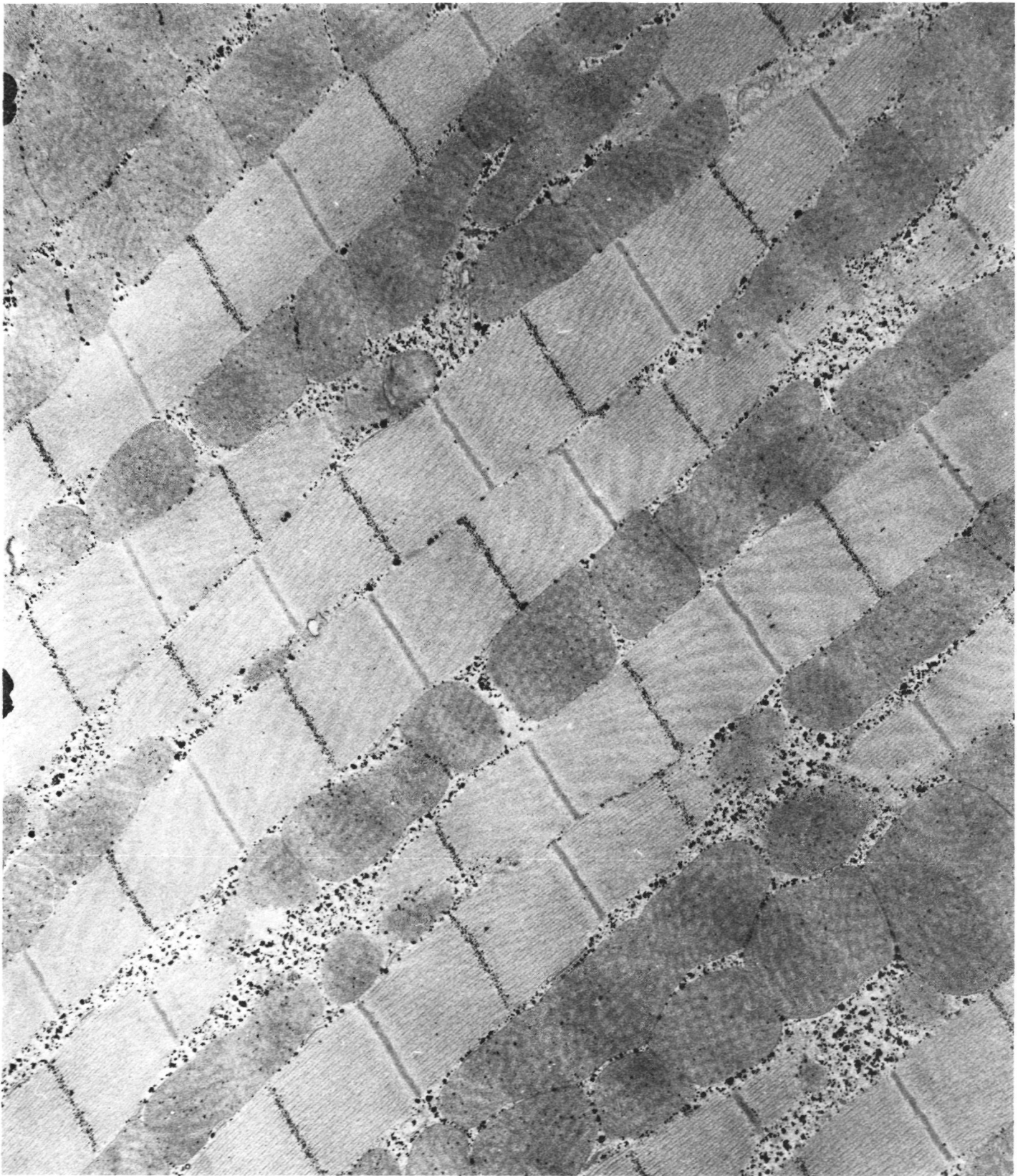


Fig. 9. Wing muscle of a 110 day old fly which was exposed to weightlessness, showing a normal appearance of myofibrils, mitochondria and glycogen granules. X11,375.

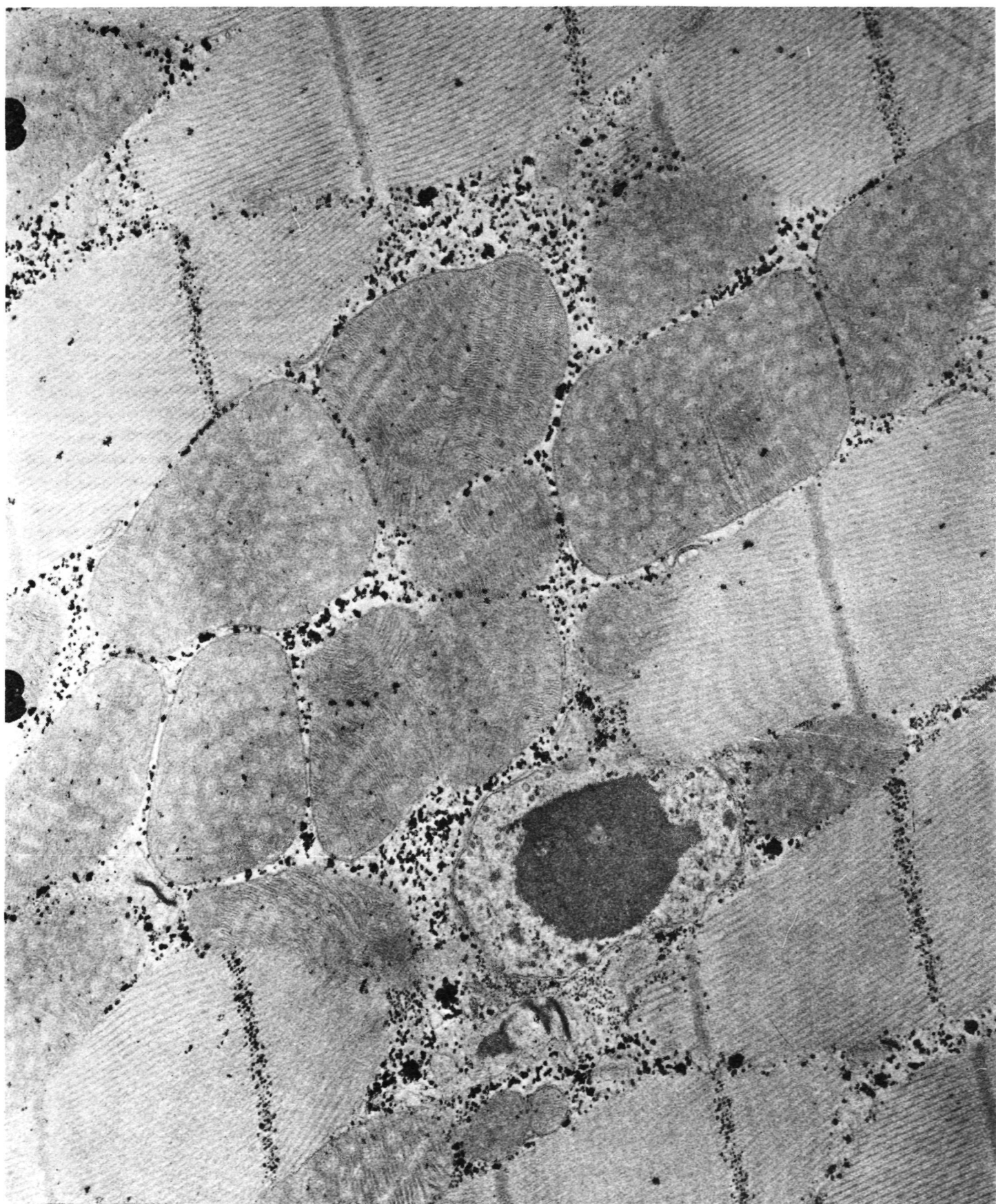


Fig. 10. High magnification electron micrograph of 110 day old Drosophila exposed to weightlessness. No atrophic changes are present. X25,650.

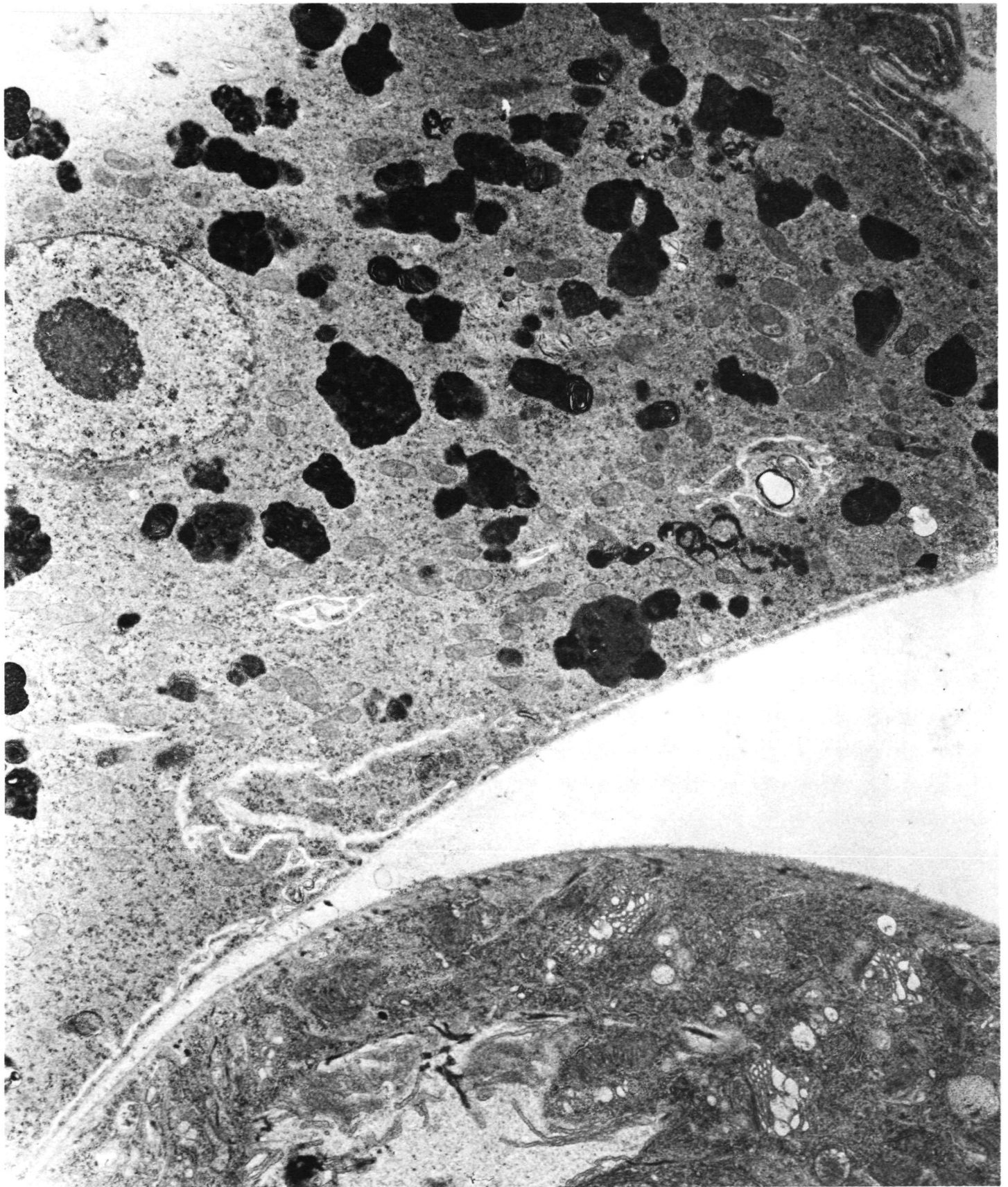


Fig. 11. Oenocytes with abundant dense bodies (age pigment) and a Malpighian tubule (lower area) in the abdomen of a 110 day old fly which was exposed to weightlessness. X11,375.

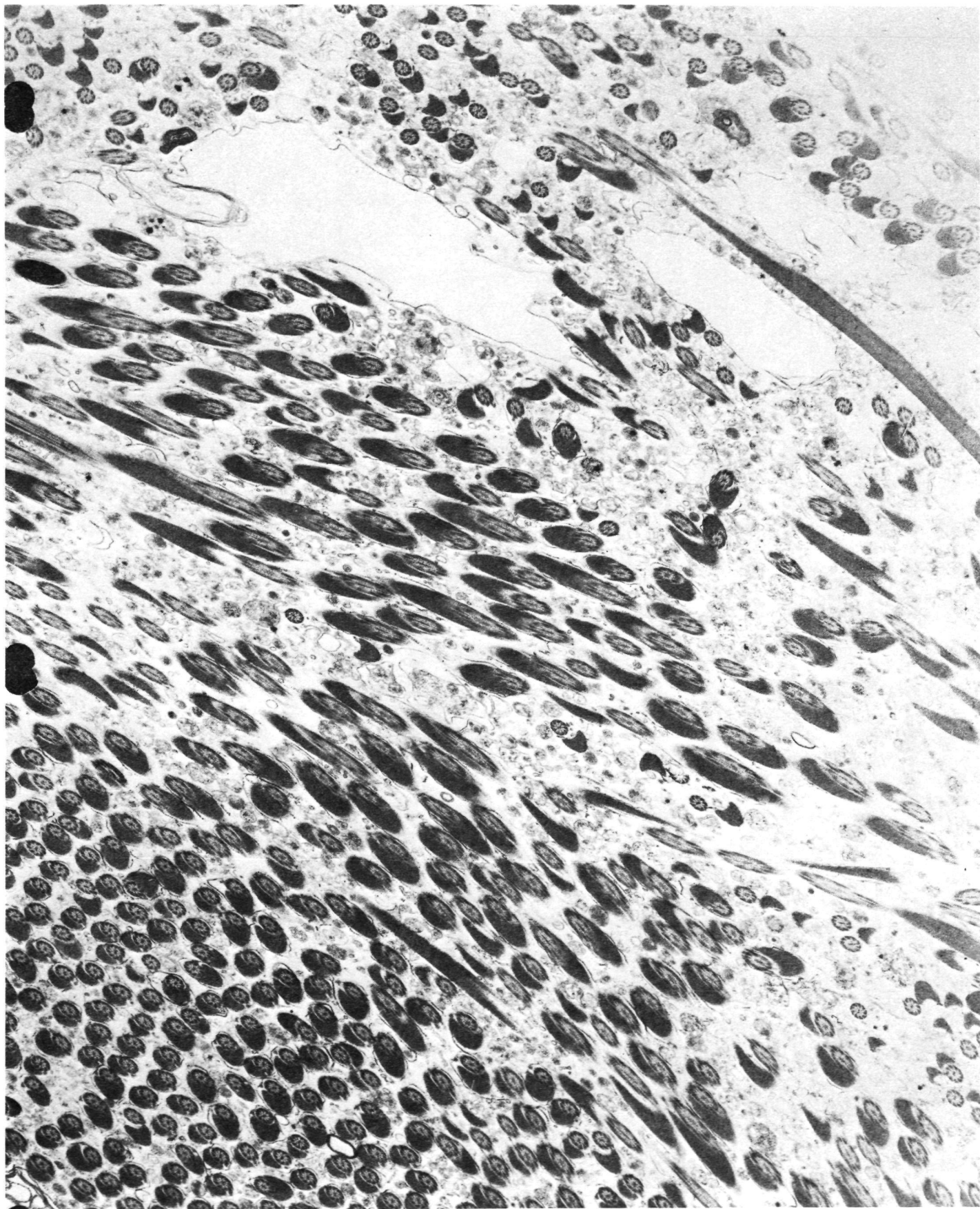


Fig. 12. Spermatozoa tails in the testes of a 110 day old fly which was exposed to weightlessness. X11,375.

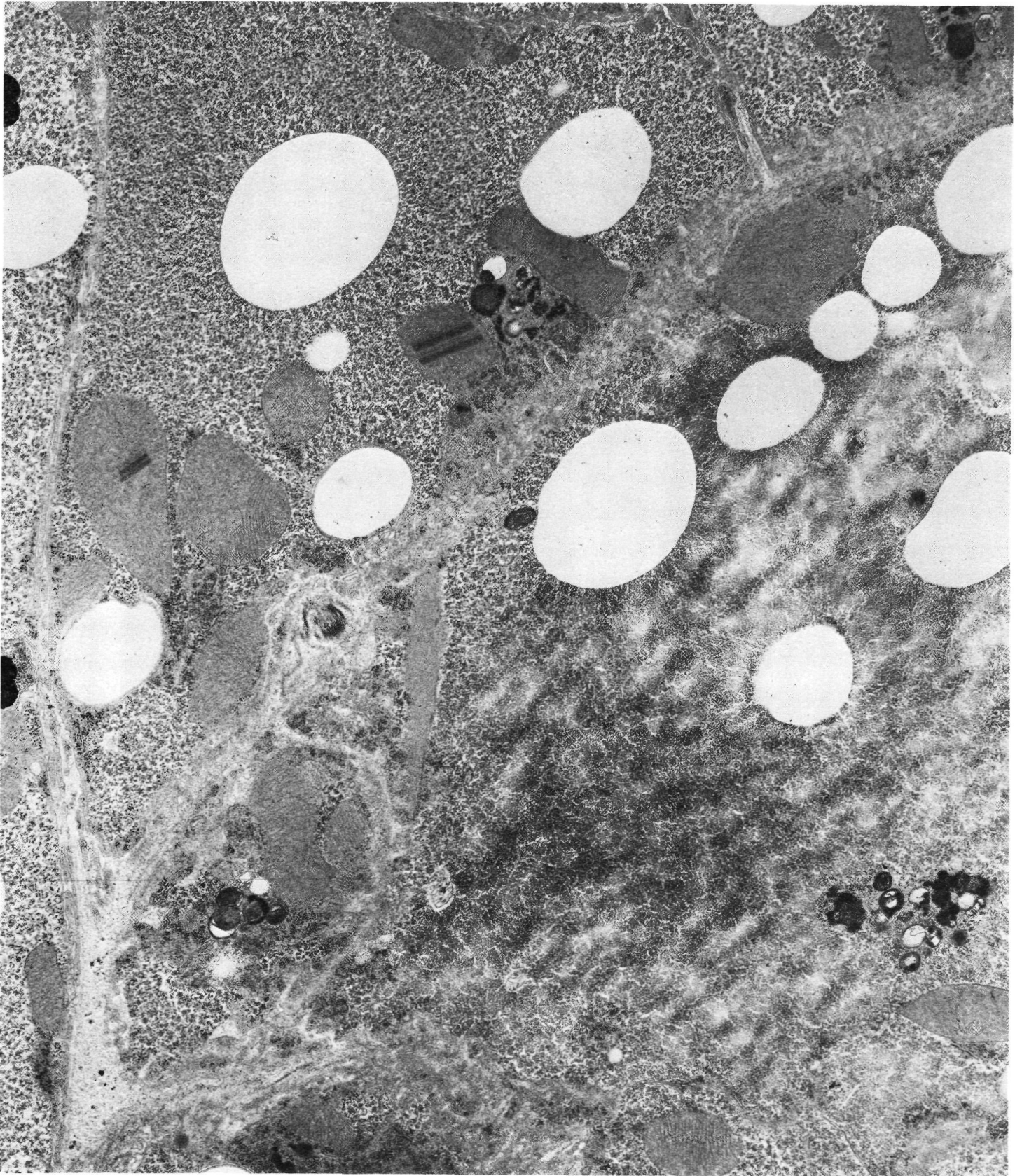


Fig. 13. Fat body cells from the abdomen of a 110 day old fly which was maintained in a centrifuge aboard the biosatellite. Lipofuscin granules, glycogen and two mitochondria containing crystalloid structures can be seen. X11,375.

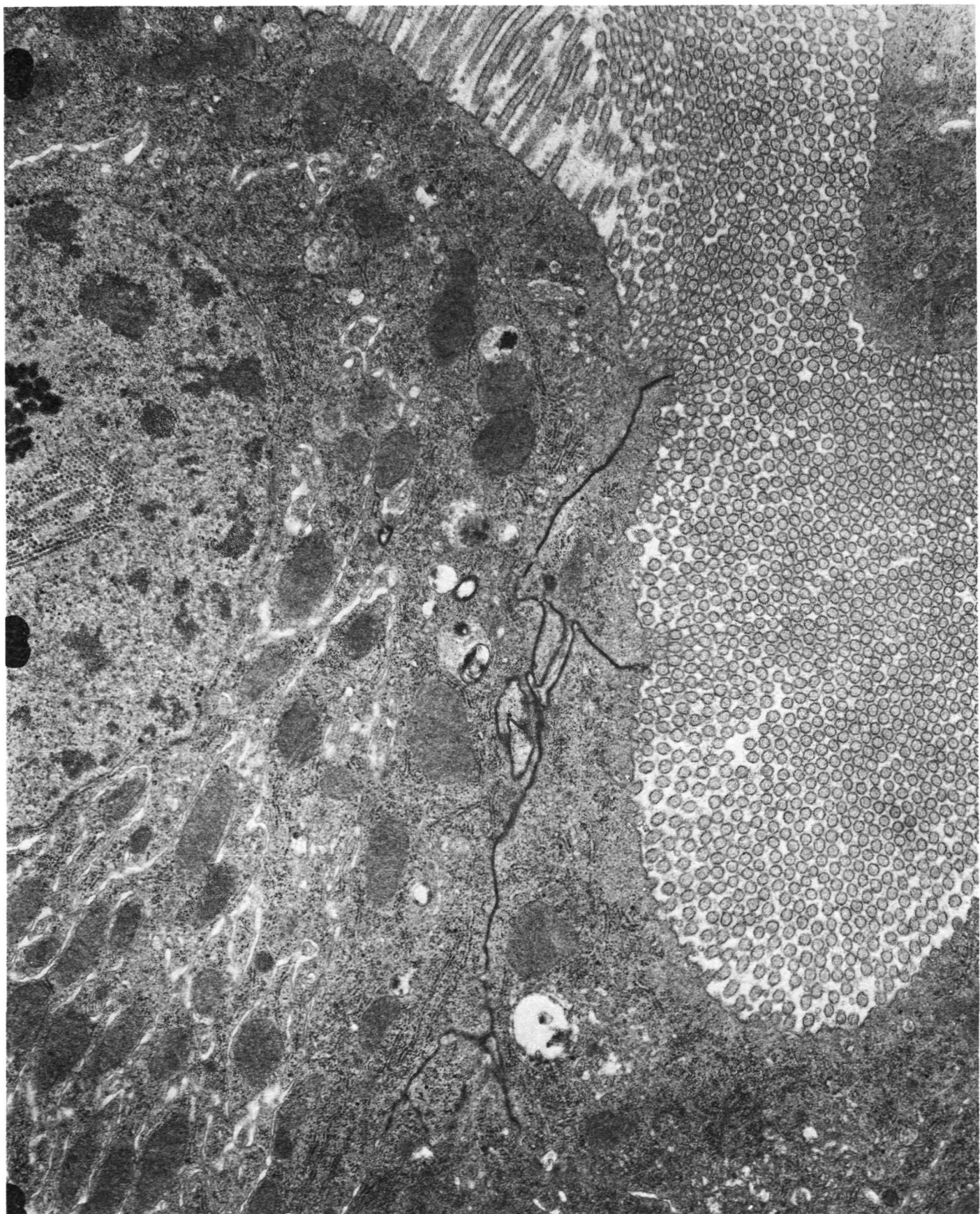


Fig. 14. Virus-like particles in the nucleus (upper left) of a midgut cell of a 110 day old flight control. X17,000.



Fig. 15. Fat body cells (left) and midgut (right) containing lipofuscin granules. The amount of pigment in the tissues of this 110 day old flight control is approximately the same than in D. melanogaster Oregon R of similar age. X11,375.

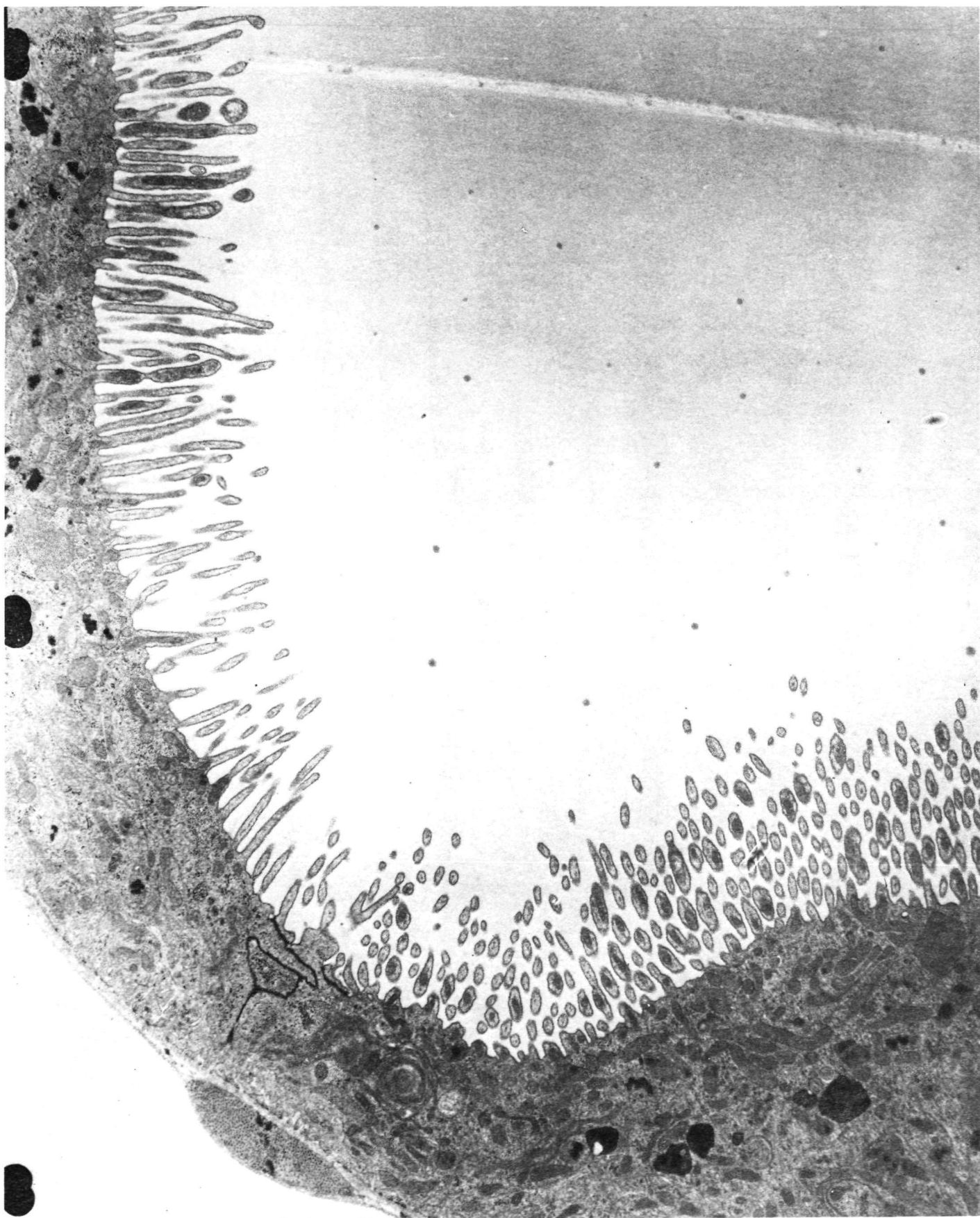


Fig. 16. Lipofuscin granules in the Malpighian tubules of a 110 day old flight control. X11,375.

Table 6: Life span of "Kosmos"-flown Drosophila

	♂				♀		
	Number	Mean life span (days)	± S.E.		Number	Mean life span (days)	± S.E.
Maintained at ~1 g (centrifuge)	31	102.6	5.4		49	109.7	3.7
Exposed to zero g	29	105.2	6.6		61	105.0	2.7

muscle atrophy, if effective flying is rendered difficult or impossible in the absence of gravity. However, our observations on glycogen content and muscle fine structure have not revealed changes similar to those described by Ilyin et al. (5) in their report on the effects of zero g on the leg muscles of rats exposed to weightlessness.

As regards the aging process, our data do not show any significant difference between the flies exposed to weightlessness and the flight controls, except for a slight reduction in the amount of age pigment of the midgut and the Malpighian tubules.

A comparison of the space-flown Drosophila with the ground controls documents the fact that the flies resisted extremely well the stresses of the "Cosmos" flight. The only detrimental effect of the flight seemed to be a decrement in the negative geotaxis and mating competence. This was particularly evident for the mating latency, that was almost double for the "Cosmos" flies than for the ground synchronous controls. It is likely that this decreased mating ability was the result of injury to the wing structures (which play a crucial role in Drosophila courtship), as the consequence of the accelerations or of other stresses unrelated to weightlessness.

In summary, our data provide very conclusive evidence about the lack of effects of weightlessness on insect development, since the flies exposed to zero g were practically identical to the flight controls as regards every parameter investigated. With respect to the possible influence of weightlessness on the aging process, we can not be so definitive. It seems to us that adult Drosophila should spend in weightlessness at least one third of its normal life span, in order to provide more conclusive evidence on this problem than has been possible to obtain by this preliminary research.

ACKNOWLEDGEMENTS:

We wish to express our gratitude to Dr. D.D. Feller and Dr. R.C. Simmonds for their invaluable help in making this study possible.

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1. Report No. NASA TM-78525		2. Government Accession No.		3. Recipient's Catalog No.	
4. Title and Subtitle FINAL REPORTS OF U.S. EXPERIMENTS FLOWN ON THE SOVIET SATELLITE COSMOS 782				5. Report Date	
				6. Performing Organization Code	
7. Author(s) Susan N. Rosenzweig* and Kenneth A. Souza				8. Performing Organization Report No. A-7612	
9. Performing Organization Name and Address NASA - Ames Research Center Moffett Field, Calif. 94035				10. Work Unit No. 199-91	
				11. Contract or Grant No.	
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration Washington, D.C. 20546				13. Type of Report and Period Covered Technical Memorandum	
				14. Sponsoring Agency Code	
15. Supplementary Notes *Northrop Services, Inc., 500 East Orangethorpe Avenue Anaheim, Calif. 92801					
16. Abstract On November 25, 1975, the Soviet Union launched Cosmos 782, an unmanned spacecraft carrying biology and physics experiments from seven countries, including the Soviet Union and the United States. The Cosmos 782 mission marked the first time the Soviet Union had flown U.S. experiments aboard one of her aircraft. The flight, which lasted 19.5 days, differed from its predecessors, Cosmos 605 and 690, in the use of a large number of species (over 20) and the presence of an on-board centrifuge for artificial gravity. The U.S.-involved experiments used rats, fruit flies, carrot tissue and cells, fish eggs, and radiation dosimeters. The Cosmos 782 experiments in general concentrated on comparing effects of low gravity versus artificial gravity on genetics, growth, development, and aging. The rat experiments focussed on low-gravity effects on endocrine, lymphoid, blood, muscle, and bone tissues. The final reports of all U.S. experiments flown on Cosmos 782 are presented.					
17. Key Words (Suggested by Author(s)) Space biology Weightlessness Aerospace medicine			18. Distribution Statement Unlimited STAR Category - 51		
19. Security Classif. (of this report) Unclassified		20. Security Classif. (of this page) Unclassified		21. No. of Pages 416	
				22. Price* \$11.00	

